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Date: October 27, 2000

Docket No.: 2750-1316P

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing is a patent application applied for on behalf of the inventor(s) according to the provisions of 37 C.F.R. § 1.41(c), which claims priority under 35 U.S.C. § 119(e) of Provisional Application No. 60/161,993 filed on October 28, 1999.

Inventor(s): Nickolai ALEXANDROV, Maxim TROUKHAN

For: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

Enclosed are:

☒ A specification consisting of a description-94 pages, Table 1-15 pages, claims-3 pages, schematic-1 page, and abstract-1 page, totaling one hundred fourteen (114) pages

☐ (   ) sheet(s) of formal drawings

☐ Certified copy of Priority Document(s)

☒ Executed Declaration in accordance with 37 C.F.R. § 1.64 will follow

☒ A statement to establish small entity status under 37 C.F.R. § 1.9 and 37 C.F.R. § 1.27

☐ Preliminary Amendment

☒ Information Sheet

☐ Information Disclosure Statement, PTO-1449 and reference(s)

☐ Amend the specification by inserting before the first line the sentence:

--This application claims priority on provisional Application No.   filed on   , the entire contents of which are hereby incorporated by reference.--

☒ Two identical CD-Rs created using IBM-PC, MS-Windows operating system. The files contained on the CD-R are as follows:

File Name	Date of Creation	File Size
001018 Protein Domain Table	October 19, 2000	2.05 MB

☐ Amend the specification by inserting before the first line the sentence:

-- This application contains a CDR, the entire contents of which are hereby incorporated by reference. The CDR contains the following files:

File Name	Date of Creation	File Size

☒ Other: Power of Attorney regarding Small Entity Statement, ATCC Deposit receipts PTA-595, PTA-1161, PTA-1411

The filing fee has been calculated as shown below:

			LARGE ENTITY	SMALL ENTITY
BASIC FEE			$710.00	$355.00
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	20- 20 =	0	X 18 = $0.00	x 9 = $0.00
INDEPENDENT CLAIMS	3- 3 =	0	x 80 = $0.00	x 40 = $0.00
☐ MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $270.00	+ $135.00
		TOTAL	$0.00	$355.00

☒ The application transmitted herewith is filed in accordance with 37 C.F.R. § 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed declaration together with the surcharge will be forwarded in due course.

☐ A check in the amount of   to cover the filing fee is enclosed.

☒ Please charge Deposit Account No. 50-1055 in the amount of $355.00 to cover the filing fee. A triplicate copy of this transmittal form is enclosed.

☒ Please send correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP - **Customer No. 2292**
P.O. Box 747
Falls Church, VA 22040-0747
Telephone: (703) 205-8000

If the Primary Deposit Account No. 50-1055 is deficient and non-payment will result in a loss of rights, the Commissioner is hereby authorized in this, concurrent and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _M&/Null #36,623_____
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RCS/DRN/JRK
2750-1316P

Attachments

(Rev. 04/19/2000)

3

Applicant, Patentee, or Identifier: N. ALEXANDROV et al.

Application or Patent No.: NEW Patent Application

Filed or Issued: October 27, 2000

Title: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

I hereby state that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN CERES, INC.

ADDRESS OF SMALL BUSINESS CONCERN 3007 Malibu Canyon Road Malibu, CA 90265

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 37 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

☒ the specification filed herewith with title as listed above.

☐ the application identified above.

☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:

☒ no such person, concern, or organization exists.

☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is not longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Mark J. Nuell (Reg. No. 36, 623)

TITLE IN ORGANIZATION OF PERSON SIGNING Legal Representative of CERES, INC.

ADDRESS OF PERSON SIGNING Birch, Stewart, Kolasch and Birch, LLP.

P.O. Box 747 Falls Church, VA 22040-0747

SIGNATURE _M. J. Nuell_   DATE October 27, 2000

Rev. 10/12/1998

ATCC

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Ceres, Inc.
Attn: Bhuvana Gopalakrishnan
3007 Malibu Canyon Road
Malibu, CA 90265

Deposited on Behalf of: Ceres, Incorporated

Identification Reference by Depositor: **Patent Deposit Designation**

Escherichia coli LIBA021800   PTA-1411

The deposit was accompanied by: __ a scientific description X a proposed taxonomic description indicated above.

The deposit was received <u>February 23, 2000</u> by this International Depository Authority and has been accepted.

AT YOUR REQUEST: <u>X</u>   We <u>will</u> inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested <u>March 1, 2000</u>. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara E. Coupé, Administrator, Patent Depository   Date: <u>March 1, 2000</u>

cc: Raymond Stewart, Esq. (Ref. Docket 80090.101)

SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

This application claims priority under 35 USC §119(e), §119(a-d) and §120 of the following applications, the entire contents of which are hereby incorporated by reference:

Country	Filing Date	Attorney No.	Client No.	Application No.
United States	10/28/1999	2750-603P	80154.002	60/161,993

5

This application contains a CDR, the entire contents of which are hereby incorporated by reference. The CDR contains the following files:

File Name	Date of Creation	File Size
001018 Protein Domain Table	October 19, 2000	2.05 MB

FIELD OF THE INVENTION

10   The present invention relates to isolated polynucleotides that represent a complete gene, or a fragment thereof, that is expressed. In addition, the present invention relates to the polypeptide or protein corresponding to the coding sequence of these polynucleotides. The present invention also relates to isolated polynucleotides that represent regulatory regions of genes. The present invention also relates to isolated polynucleotides that represent

15   untranslated regions of genes. The present invention further relates to the use of these isolated polynucleotides and polypeptides and proteins.

DESCRIPTION OF THE RELATED ART

Efforts to map and sequence the genome of a number of organisms are in progress; a few complete genome sequences, for example those of *E. coli* and *Saccharomyces cerevisiae* are

20   known (Blattner et al., *Science* 277:1453 (1997); Goffeau et al., *Science* 274:546 (1996)). The complete genome of a multicellular organism, *C. elegans*, has also been sequenced (See, the *C. elegans* Sequencing Consortium, *Science* 282:2012 (1998)). To date, no complete genome of a plant has been sequenced, nor has a complete cDNA complement of any plant been sequenced.

25   ## SUMMARY OF THE INVENTION

The present invention comprises polynucleotides, such as complete cDNA sequences and/or sequences of genomic DNA encompassing complete genes, fragments of genes, and/or regulatory elements of genes and/or regions with other functions and/or intergenic regions, hereinafter collectively referred to as Sequence-Determined DNA Fragments (SDFs), from

different plant species, particularly corn, wheat, soybean, rice and *Arabidopsis thaliana,* and other plants and or mutants, variants, fragments or fusions of said SDFs and polypeptides or proteins derived therefrom. In some instances, the SDFs span the entirety of a protein-coding segment. In some instances, the entirety of an mRNA is represented. Other objects of the

5   invention that are also represented by SDFs of the invention are control sequences, such as, but not limited to, promoters. Complements of any sequence of the invention are also considered part of the invention.

Other objects of the invention are polynucleotides comprising exon sequences, polynucleotides comprising intron sequences, polynucleotides comprising introns together with

10   exons, intron/exon junction sequences, 5' untranslated sequences, and 3' untranslated sequences of the SDFs of the present invention. Polynucleotides representing the joinder of any exons described herein, in any arrangement, for example, to produce a sequence encoding any desirable amino acid sequence are within the scope of the invention.

The present invention also resides in probes useful for isolating and identifying nucleic

15   acids that hybridize to an SDF of the invention. The probes can be of any length, but more typically are 12-2000 nucleotides in length; more typically, 15 to 200 nucleotides long; even more typically, 18 to 100 nucleotides long.

Yet another object of the invention is a method of isolating and/or identifying nucleic acids using the following steps:

20   (a)   contacting a probe of the instant invention with a polynucleotide sample under conditions that permit hybridization and formation of a polynucleotide duplex; and

(b)   detecting and/or isolating the duplex of step (a).

The conditions for hybridization can be from low to moderate to high stringency conditions. The sample can include a polynucleotide having a sequence unique in a plant

25   genome. Probes and methods of the invention are useful, for example, without limitation, for mapping of genetic traits and/or for positional cloning of a desired fragment of genomic DNA.

Probes and methods of the invention can also be used for detecting alternatively spliced messages within a species. Probes and methods of the invention can further be used to detect or isolate related genes in other plant species using genomic DNA (gDNA) and/or cDNA libraries.

30   In some instances, especially when longer probes and low to moderate stringency hybridization conditions are used; the probe will hybridize to a plurality of cDNA and/or gDNA sequences of a plant. This approach is useful for isolating representatives of gene families which are identifiable by possession of a common functional domain in the gene product or which have

3

common cis-acting regulatory sequences. This approach is also useful for identifying orthologous genes from other organisms.

The present invention also resides in constructs for modulating the expression of the genes comprised of all or a fragment of an SDF. The constructs comprise all or a fragment of

5 the expressed SDF, or of a complementary sequence. Examples of constructs include ribozymes comprising RNA encoded by an SDF or by a sequence complementary thereto, antisense constructs, constructs comprising coding regions or parts thereof, constructs comprising promoters, introns, untranslated regions, scaffold attachment regions, methylating regions, enhancing or reducing regions, DNA and chromatin conformation modifying

10 sequences, etc. Such constructs can be constructed using viral, plasmid, bacterial artificial chromosomes (BACs), plasmid artificial chromosomes (PACs), autonomous plant plasmids, plant artificial chromosomes or other types of vectors and exist in the plant as autonomous replicating sequences or as DNA integrated into the genome. When inserted into a host cell the construct is, preferably, functionally integrated with, or operatively linked to, a

15 heterologous polynucleotide. For instance, a coding region from an SDF might be operably linked to a promoter that is functional in a plant.

The present invention also resides in host cells, including bacterial or yeast cells or plant cells, and plants that harbor constructs such as described above. Another aspect of the invention relates to methods for modulating expression of specific genes in plants by expression of the

20 coding sequence of the constructs, by regulation of expression of one or more endogenous genes in a plant or by suppression of expression of the polynucleotides of the invention in a plant. Methods of modulation of gene expression include without limitation (1) inserting into a host cell additional copies of a polynucleotide comprising a coding sequence; (2) modulating an endogenous promoter in a host cell; (3) inserting antisense or ribozyme constructs into a host

25 cell and (4) inserting into a host cell a polynucleotide comprising a sequence encoding a variant , fragment, or fusion of the native polypeptides of the instant invention.

BRIEF DESCRIPTION OF THE TABLES

In TABLE 1, the format of the data is as follows:

30 In Table 1, sequence data are presented in the form of annotation of a reference sequence. The format is shown below. The reference sequence is shown at the top of the annotation file as a 7 digit sequence number preceded by >" (e.g. >5019261). The sequence identifier is a gi" number that identifies a specific DNA sequence in the publically accessible BLAST Databases on the NCBI FTP web site (accessible at ncbi.nlm.gov/blast).

In particular, the nt.Z" nucleotide sequence data base at the NCBI FTP site utilizes the gi"
identifiers to assign by NCBI a unique identifier for each sequence in the databases, thereby
providing a non-redundant database for sequences from various data bases, including
GenBank, EMBL, DDBJ (DNA Database of Japan) and PDB (Brookhaven Protein Data

5 Bank). Thus, the line in TABLE 1 beginning with sequence number identifies the unique gi"
identifier followed by the corresponding GenBank (gb) accession number and locus. The gi
identifier number is followed by a /" and if present a number cataloging a corresponding
clone identifier number, which denotes a cDNA sequence that aligns with the genomic DNA
sequence. The reference sequence number is followed on the next line by data regarding the

10 length of the sequence (len") and the number of exons found in the sequence by the analysis
program (nex").

The annotation data are presented in columns; the leftmost column identifies the position of
the putative exon in the gene as initial (init"), internal (intr") or terminal (term"). Genes
considered composed of a single exon are denoted sngl". The next column describes the

15 position in the nucleotide sequence beginning the exon (start") and the next column
describes the position in the nucleotide sequence ending the exon (stop"). The direction of
the gene is indicated in the next column, +" indicating 5' - 3' in the direction presented in
the database, - indicating the opposite orientation. The gene number" is given in the final
column. Exons having the same gene number are grouped in the order shown to create the

20 relevant coding sequence.

>5019261 ⇐ This is the gi number of the public sequence

len = 97208   nex = 121

⇑   ⇑

Length   Number exons

5 of public sequence

Exon Type	Start	Stop	Direction	Gene Number
⇓	⇓	⇓	⇓	⇓
Sngl	602	778	+	0
Sngl	990	1316	+	1
Sngl	2356	2691	+	2
Sngl	4634	4735	+	3
Sngl	4973	5092	+	4
Sngl	5746	5874	+	5
Init	8119	8798	+	6
Term	9284	9518	+	6
Init	10827	11150	+	7
Term	11294	11335	+	7
Sngl	12655	12825	+	8
Sngl	13303	13596	+	9
Sngl	18654	18782	+	10
Sngl	19880	20086	+	11
Init	21476	21539	+	12
Intr	21647	21802	+	12
Term	23488	23567	+	12
Init	25035	25133	+	13
Intr	25466	25589	+	13
Intr	25677	25786	+	13
Intr	25899	25962	+	13
Intr	26045	26109	+	13
Intr	26188	26253	+	13
Term	26350	26448	+	13
Sngl	27671	27793	+	14
Sngl	29126	29299	+	15
Sngl	30266	30364	+	16
Sngl	31717	31929	+	17
Sngl	32102	32209	+	18
Sngl	32450	32548	+	19

The line numbers in the left margin: 10, 15, 20, 25, 30, 35, 40

6

Sngl	32634	32726	+	20
Init	35603	35743	+	21
Term	35829	36185	+	21
Init	36954	37098	+	22
Term	38100	38158	+	22
Init	39635	39944	+	23
Intr	40242	40372	+	23
Intr	40462	40695	+	23
Intr	40815	41070	+	23
Intr	41176	41255	+	23
Intr	42212	42419	+	23
Intr	42940	43070	+	23
Intr	43177	43410	+	23
Intr	43580	43835	+	23
Intr	46672	46715	+	23
Intr	48334	48532	+	23

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to (I) polynucleotides and methods of use thereof, such as

IA.   Probes, Primers and Substrates;

IB.   Methods of Detection and Isolation;

    B.1.   Hybridization;

    B.2.   Methods of Mapping;

    B.3.   Southern Blotting;

    B.4.   Isolating cDNA from Related Organisms;

    B.5.   Isolating and/or Identifying Orthologous Genes

IC.   Methods of Inhibiting Gene Expression

    C.1.   Antisense

    C.2.   Ribozyme Constructs;

    C.3.   Chimeraplasts;

    C.4   Co-Suppression;

    C.5.   Transcriptional Silencing

    C.6.   Other Methods to Inhibit Gene Expression

ID.   Methods of Functional Analysis;

IE.   Promoter Sequences and Their Use;

IF.   UTRs and/or Intron Sequences and Their Use; and

IG.   Coding Sequences and Their Use.

The invention also relates to (II) polypeptides and proteins and methods of use thereof,

such as IIA. Native Polypeptides and Proteins

5   A.1 Antibodies

             A.2 In Vitro Applications

IIB. Polypeptide Variants, Fragments and Fusions

             B.1 Variants

             B.2 Fragments

10   B.3 Fusions

The invention also includes (III) methods of modulating polypeptide production, such as

IIIA. Suppression

             A.1 Antisense

15   A.2 Ribozymes

             A.3 Co-suppression

             A.4 Insertion of Sequences into the Gene to be Modulated

             A.5 Promoter Modulation

             A.6 Expression of Genes containing Dominant-Negative Mutations

20 IIIB. Enhanced Expression

             B.1 Insertion of an Exogenous Gene

             B.2 Promoter Modulation

The invention further concerns (IV) gene constructs and vector construction, such as

25 IVA. Coding Sequences

IVB. Promoters

IVC. Signal Peptides

The invention still further relates to

30 V   Transformation Techniques

Definitions

Allelic variant   An allelic variant" is an alternative form of the same SDF, which
resides at the same chromosomal locus in the organism. Allelic variations can occur in any

5   portion of the gene sequence, including regulatory regions. Allelic variants can arise by
normal genetic variation in a population. Allelic variants can also be produced by genetic
engineering methods. An allelic variant can be one that is found in a naturally occurring
plant, including a cultivar or ecotype. An allelic variant may or may not give rise to a
phenotypic change, and may or may not be expressed. An allele can result in a detectable

10   change in the phenotype of the trait represented by the locus. A phenotypically silent allele
can give rise to a product.

Alternatively spliced messages   Within the context of the current invention,
alternatively spliced messages" refers to mature mRNAs originating from a single gene with

15   variations in the number and/or identity of exons, introns and/or intron-exon junctions.

Chimeric   The term chimeric" is used to describe genes, as defined supra, or contructs
wherein at least two of the elements of the gene or construct, such as the promoter and the
coding sequence and/or other regulatory sequences and/or filler sequences and/or complements

20   thereof, are heterologous to each other.

Constitutive Promoter: Promoters referred to herein as "constitutive promoters" actively promote
transcription under most, but not necessarily all, environmental conditions and states of
development or cell differentiation. Examples of constitutive promoters include the cauliflower

25   mosaic virus (CaMV) 35S transcript initiation region and the 1' or 2' promoter derived from
T-DNA of *Agrobacterium tumefaciens,* and other transcription initiation regions from various
plant genes, such as the maize ubiquitin-1 promoter, known to those of skill.

Coordinately Expressed: The term coordinately expressed," as used in the current

30   invention, refers to genes that are expressed at the same or a similar time and/or stage and/or
under the same or similar environmental conditions.

Domain: Domains are fingerprints or signatures that can be used to characterize protein families and/or parts of proteins. Such fingerprints or signatures can comprise conserved (1) primary sequence, (2) secondary structure, and/or (3) three-dimensional conformation. Generally, each domain has been associated with either a family of proteins or

5 motifs. Typically, these families and/or motifs have been correlated with specific *in-vitro* and/or *in-vivo* activities. A domain can be any length, including the entirety of the sequence of a protein. Detailed descriptions of the domains, associated families and motifs, and correlated activities of the polypeptides of the instant invention are described below. Usually, the polypeptides with designated domain(s) can exhibit at least one activity that is

10 exhibited by any polypeptide that comprises the same domain(s).

Endogenous The term endogenous," within the context of the current invention refers to any polynucleotide, polypeptide or protein sequence which is a natural part of a cell or

15 organisms regenerated from said cell.

Exogenous Exogenous," as referred to within, is any polynucleotide, polypeptide or protein sequence, whether chimeric or not, that is initially or subsequently introduced into the genome of an individual host cell or the organism regenerated from said host cell by any

20 means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation (of dicots - *e.g.* Salomon et al. *EMBO J.* 3:141 (1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983); of monocots, representative papers are those by Escudero et al., *Plant J.* 10:355 (1996), Ishida et al., *Nature Biotechnology* 14:745 (1996), May et al., *Bio/Technology* 13:486 (1995)), biolistic

25 methods (Armaleo et al., *Current Genetics* 17:97 1990)), electroporation, *in planta* techniques, and the like. Such a plant containing the exogenous nucleic acid is referred to here as a T_0 for the primary transgenic plant and T_1 for the first generation. The term exogenous" as used herein is also intended to encompass inserting a naturally found element into a non-naturally found location.

30

Filler sequence: As used herein, filler sequence" refers to any nucleotide sequence that is inserted into DNA construct to evoke a particular spacing between particular components such as a promoter and a coding region and may provide an additional attribute such as a restriction enzyme site.

Gene: The term "gene," as used in the context of the current invention, encompasses all regulatory and coding sequence contiguously associated with a single hereditary unit with a genetic function (see SCHEMATIC 1). Genes can include non-coding sequences that

5   modulate the genetic function that include, but are not limited to, those that specify polyadenylation, transcriptional regulation, DNA conformation, chromatin conformation, extent and position of base methylation and binding sites of proteins that control all of these. Genes comprised of "exons" (coding sequences), which may be interrupted by "introns" (non-coding sequences), encode proteins. A gene's genetic function may require only RNA

10   expression or protein production, or may only require binding of proteins and/or nucleic acids without associated expression. In certain cases, genes adjacent to one another may share sequence in such a way that one gene will overlap the other. A gene can be found within the genome of an organism, artificial chromosome, plasmid, vector, etc., or as a separate isolated entity.

15

Gene Family: "Gene family" is used in the current invention to describe a group of functionally related genes, each of which encodes a separate protein.

Heterologous sequences: "Heterologous sequences" are those that are not operatively

20   linked or are not contiguous to each other in nature. For example, a promoter from corn is considered heterologous to an *Arabidopsis* coding region sequence. Also, a promoter from a gene encoding a growth factor from corn is considered heterologous to a sequence encoding the corn receptor for the growth factor. Regulatory element sequences, such as UTRs or 3' end termination sequences that do not originate in nature from the same gene as the coding sequence

25   originates from, are considered heterologous to said coding sequence. Elements operatively linked in nature and contiguous to each other are not heterologous to each other. On the other hand, these same elements remain operatively linked but become heterologous if other filler sequence is placed between them. Thus, the promoter and coding sequences of a corn gene expressing an amino acid transporter are not heterologous to each other, but the promoter and

30   coding sequence of a corn gene operatively linked in a novel manner are heterologous.

Homologous gene   In the current invention, "homologous gene" refers to a gene that shares sequence similarity with the gene of interest. This similarity may be in only a fragment of the sequence and often represents a functional domain such as, examples including without

limitation a DNA binding domain, a domain with tyrosine kinase activity, or the like. The functional activities of homologous genes are not necessarily the same.

Inducible Promoter   An inducible promoter" in the context of the current invention

5   refers to a promoter which is regulated under certain conditions, such as light, chemical concentration, protein concentration, conditions in an organism, cell, or organelle, etc. A typical example of an inducible promoter, which can be utilized with the polynucleotides of the present invention, is PARSK1, the promoter from the *Arabidopsis* gene encoding a serine-threonine kinase enzyme, and which promoter is induced by dehydration, abscissic acid and sodium

10   chloride (Wang and Goodman, *Plant J.* 8:37 (1995)) Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light.

15   Intergenic region   Intergenic region," as used in the current invention, refers to nucleotide sequence occurring in the genome that separates adjacent genes.

Mutant gene   In the current invention, mutant" refers to a heritable change in DNA sequence at a specific location. Mutants of the current invention may or may not have an

20   associated identifiable function when the mutant gene is transcribed.

Orthologous Gene   In the current invention orthologous gene" refers to a second gene that encodes a gene product that performs a similar function as the product of a first gene. The orthologous gene may also have a degree of sequence similarity to the first gene. The

25   orthologous gene may encode a polypeptide that exhibits a degree of sequence similarity to a polypeptide corresponding to a first gene. The sequence similarity can be found within a functional domain or along the entire length of the coding sequence of the genes and/or their corresponding polypeptides.

30   Percentage of sequence identity   "Percentage of sequence identity," as used herein, is determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences.

The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence

5 identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and

10 TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment. Typically, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. The term "substantial sequence identity" between polynucleotide or polypeptide sequences

15 refers to polynucleotide or polypeptide comprising a sequence that has at least 80% sequence identity, preferably at least 85%, more preferably at least 90% and most preferably at least 95%, even more preferably, at least 96%, 97%, 98% or 99% sequence identity compared to a reference sequence using the programs.

20 Plant Promoter   A plant promoter" is a promoter capable of initiating transcription in plant cells and can drive or facilitate transcription of a fragment of the SDF of the instant invention or a coding sequence of the SDF of the instant invention. Such promoters need not be of plant origin. For example, promoters derived from plant viruses, such as the CaMV35S promoter or from *Agrobacterium tumefaciens* such as the T-DNA promoters, can be plant

25 promoters. A typical example of a plant promoter of plant origin is the maize ubiquitin-1 (ubi-1)promoter known to those of skill.

30 Promoter: The term "promoter," as used herein, refers to a region of sequence determinants located upstream from the start of transcription of a gene and which are involved in recognition and binding of RNA polymerase and other proteins to initiate and modulate transcription. A basal promoter is the minimal sequence necessary for assembly of a transcription complex required for transcription initiation. Basal promoters frequently include a

TATA box" element usually located between 15 and 35 nucleotides upstream from the site of initiation of transcription. Basal promoters also sometimes include a CCAAT box" element (typically a sequence CCAAT) and/or a GGGCG sequence, usually located between 40 and 200 nucleotides, preferably 60 to 120 nucleotides, upstream from the start site of transcription.

5

Public sequence: The term public sequence," as used in the context of the instant application, refers to any sequence that has been deposited in a publicly accessible database. This term encompasses both amino acid and nucleotide sequences. Such sequences are publicly accessible, for example, on the BLAST databases on the NCBI FTP web site

10   (accessible at ncbi.nlm.gov/blast). The database at the NCBI GTP site utilizes gi" numbers assigned by NCBI as a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequence from various databases, including GenBank, EMBL, DBBJ, (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank).

15

Regulatory Sequence   The term regulatory sequence," as used in the current invention, refers to any nucleotide sequence that influences transcription or translation initiation and rate, and stability and/or mobility of the transcript or polypeptide product. Regulatory sequences include, but are not limited to, promoters, promoter control elements,

20   protein binding sequences, 5' and 3' UTRs, transcriptional start site, termination sequence, polyadenylation sequence, introns, certain sequences within a coding sequence, etc.

Related Sequences: Related sequences" refer to either a polypeptide or a nucleotide sequence that exhibits some degree of sequence similarity with a sequence described in Table

25   1.

Scaffold Attachment Region (SAR)   As used herein, scaffold attachment region" is a DNA sequence that anchors chromatin to the nuclear matrix or scaffold to generate loop domains that can have either a transcriptionally active or inactive structure (Spiker and Thompson

30   (1996) Plant Physiol. 110: 15-21).

Sequence-determined DNA fragments (SDFs)   Sequence-determined DNA fragments" as used in the current invention are isolated sequences of genes, fragments of genes,

intergenic regions or contiguous DNA from plant genomic DNA or cDNA or RNA the sequence of which has been determined.

Signal Peptide   A signal peptide" as used in the current invention is an amino acid
5   sequence that targets the protein for secretion, for transport to an intracellular compartment or organelle or for incorporation into a membrane. Signal peptides are indicated in the tables and a more detailed description located below.

Specific Promoter   In the context of the current invention,   specific promoters" refers to a
10   subset of inducible promoters that have a high preference for being induced in a specific tissue or cell and/or at a specific time during development of an organism. By   high preference" is meant at least 3-fold, preferably 5-fold, more preferably at least 10-fold still more preferably at least 20-fold, 50-fold or 100-fold increase in transcription in the desired tissue over the transcription in any other tissue. Typical examples of temporal and/or tissue
15   specific promoters of plant origin that can be used with the polynucleotides of the present invention, are: PTA29, a promoter which is capable of driving gene transcription specifically in tapetum and only during anther development (Koltonow et al., *Plant Cell* 2:1201 (1990); RCc2 and RCc3, promoters that direct root-specific gene transcription in rice (Xu et al., *Plant Mol. Biol.* 27:237 (1995); TobRB27, a root-specific promoter from tobacco (Yamamoto et al., *Plant*
20   *Cell* 3:371 (1991)). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues or organs, such as root, ovule, fruit, seeds, or flowers. Other suitable promoters include those from genes encoding storage proteins or the lipid body membrane protein, oleosin. A few root-specific promoters are noted above.

25   Stringency   "Stringency" as used herein is a function of probe length, probe composition (G + C content), and salt concentration, organic solvent concentration, and temperature of hybridization or wash conditions. Stringency is typically compared by the parameter T_m, which is the temperature at which 50% of the complementary molecules in the hybridization are hybridized, in terms of a temperature differential from T_m. High stringency conditions are those
30   providing a condition of T_m - 5°C to T_m - 10°C. Medium or moderate stringency conditions are those providing T_m - 20°C to T_m - 29°C. Low stringency conditions are those providing a condition of T_m - 40°C to T_m - 48°C. The relationship of hybridization conditions to T_m (in °C) is expressed in the mathematical equation

$$T_m = 81.5 - 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N) \quad (1)$$

where N is the length of the probe. This equation works well for probes 14 to 70 nucleotides in length that are identical to the target sequence. The equation below for T_m of DNA-DNA hybrids is useful for probes in the range of 50 to greater than 500 nucleotides, and for conditions

5   that include an organic solvent (formamide).

$$T_m = 81.5 + 16.6 \log \{[Na^+]/(1+0.7[Na^+])\} + 0.41(\%G+C) - 500/L \ 0.63(\%formamide) \quad (2)$$

where L is the length of the probe in the hybrid. (P. Tijessen, Hybridization with Nucleic Acid Probes" in Laboratory Techniques in Biochemistry and Molecular Biology, P.C. vand der Vliet, ed., c. 1993 by Elsevier, Amsterdam.) The T_m of equation (2) is affected by the

10   nature of the hybrid; for DNA-RNA hybrids T_m is 10-15°C higher than calculated, for RNA-RNA hybrids T_m is 20-25°C higher. Because the T_m decreases about 1 °C for each 1% decrease in homology when a long probe is used (Bonner et al., *J. Mol. Biol.* 81:123 (1973)), stringency conditions can be adjusted to favor detection of identical genes or related family members.

15   Equation (2) is derived assuming equilibrium and therefore, hybridizations according to the present invention are most preferably performed under conditions of probe excess and for sufficient time to achieve equilibrium. The time required to reach equilibrium can be shortened by inclusion of a hybridization accelerator such as dextran sulfate or another high volume polymer in the hybridization buffer.

20   Stringency can be controlled during the hybridization reaction or after hybridization has occurred by altering the salt and temperature conditions of the wash solutions used. The formulas shown above are equally valid when used to compute the stringency of a wash solution. Preferred wash solution stringencies lie within the ranges stated above; high stringency is 5-8°C below T_m, medium or moderate stringency is 26-29°C below T_m and low

25   stringency is 45-48°C below T_m.

Substantially free of   A composition containing A is substantially free of   B when at least 85% by weight of the total A+B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A+B in the composition, more preferably at least

30   about 95% or even 99% by weight. For example, a plant gene or DNA sequence can be considered substantially free of other plant genes or DNA sequences.

Translational start site   In the context of the current invention, a translational start site" is usually an ATG in the cDNA transcript, more usually the first ATG. A single cDNA, however, may have multiple translational start sites.

5

Transcription start site   Transcription start site" is used in the current invention to describe the point at which transcription is initiated. This point is typically located about 25 nucleotides downstream from a TFIID binding site, such as a TATA box. Transcription can initiate at one or more sites within the gene, and a single gene may have multiple transcriptional

10   start sites, some of which may be specific for transcription in a particular cell-type or tissue.

Untranslated region (UTR)   A UTR" is any contiguous series of nucleotide bases that is transcribed, but is not translated. These untranslated regions may be associated with particular functions such as increasing mRNA message stability. Examples of UTRs include, but are not limited to polyadenylation signals, terminations sequences, sequences located

15   between the transcriptional start site and the first exon (5' UTR) and sequences located between the last exon and the end of the mRNA (3' UTR).

Variant: The term variant" is used herein to denote a polypeptide or protein or polynucleotide molecule that differs from others of its kind in some way. For example,

20   polypeptide and protein variants can consist of changes in amino acid sequence and/or charge and/or post-translational modifications (such as glycosylation, etc).

25

DETAILED DESCRIPTION OF THE INVENTION

I.   Polynucleotides

Exemplified SDFs of the invention represent fragments of the genome of corn, wheat, rice, soybean or *Arabidopsis* and/or represent mRNA expressed from that genome. The isolated nucleic acid of the invention also encompasses corresponding fragments of the genome and/or

30   cDNA complement of other organisms as described in detail below.

Polynucleotides of the invention can be isolated from polynucleotide libraries using primers comprising sequence similar to those described by Table 1. See, for example, the methods described in Sambrook et al., supra.

Alternatively, the polynucleotides of the invention can be produced by chemical

5 synthesis. Such synthesis methods are described below.

It is contemplated that the nucleotide sequences presented herein may contain some small percentage of errors. These errors may arise in the normal course of determination of nucleotide sequences. Sequence errors can be corrected by obtaining seeds deposited under the accession numbers cited herein, propagating them, isolating genomic DNA or appropriate

10 mRNA from the resulting plants or seeds thereof, amplifying the relevant fragment of the genomic DNA or mRNA using primers having a sequence that flanks the erroneous sequence, and sequencing the amplification product.

I.A.   Probes, Primers and Substrates

SDFs of the invention can be applied to substrates for use in array applications such

15 as, but not limited to, assays of global gene expression, for example under varying conditions of development, growth conditions. The arrays can also be used in diagnostic or forensic methods (WO95/35505, US 5,445,943 and US 5,410,270).

Probes and primers of the instant invention will hybridize to a polynucleotide comprising a sequence in Table 1. Though many different nucleotide sequences can encode

20 an amino acid sequence, the sequences of Table 1 are generally preferred for encoding polypeptides of the invention. However, the sequence of the probes and/or primers of the instant invention need not be identical to those in Table 1 or the complements thereof. For example, some variation in probe or primer sequence and/or length can allow additional family members to be detected, as well as orthologous genes and more taxonomically distant

25 related sequences. Similarly, probes and/or primers of the invention can include additional nucleotides that serve as a label for detecting the formed duplex or for subsequent cloning purposes.

Probe length will vary depending on the application. For use as primers, probes are 12-40 nucleotides, preferably 18-30 nucleotides long. For use in mapping, probes are

30 preferably 50 to 500 nucleotides, preferably 100-250 nucleotides long. For Southern hybridizations, probes as long as several kilobases can be used as explained below.

The probes and/or primers can be produced by synthetic procedures such as the triester method of Matteucci et al. *J. Am. Chem. Soc.* 103:3185(1981); or according to Urdea

et al. *Proc. Natl. Acad.* 80:7461 (1981) or using commercially available automated oligonucleotide synthesizers.

5   I.B.   <u>Methods of Detection and Isolation</u>

The polynucleotides of the invention can be utilized in a number of methods known to those skilled in the art as probes and/or primers to isolate and detect polynucleotides, including, without limitation: Southerns, Northerns, Branched DNA hybridization assays, polymerase chain reaction, and microarray assays, and variations thereof. Specific methods

10   given by way of examples, and discussed below include:

Hybridization

Methods of Mapping

Southern Blotting

Isolating cDNA from Related Organisms

15   Isolating and/or Identifying Orthologous Genes.

Also, the nucleic acid molecules of the invention can used in other methods, such as high density oligonucleotide hybridizing assays, described, for example, in U.S. Pat. Nos. 6,004,753; 5,945,306; 5,945,287; 5,945,308; 5,919,686; 5,919,661; 5,919,627; 5,874,248; 5,871,973; 5,871,971; and 5,871,930; and PCT Pub. Nos. WO 9946380; WO 9933981; WO

20   9933870; WO 9931252; WO 9915658; WO 9906572; WO 9858052; WO 9958672; and WO 9810858.

B.1.   Hybridization

The isolated SDFs of Table 1 of the present invention can be used as probes and/or

25   primers for detection and/or isolation of related polynucleotide sequences through hybridization. Hybridization of one nucleic acid to another constitutes a physical property that defines the subject SDF of the invention and the identified related sequences. Also, such hybridization imposes structural limitations on the pair. A good general discussion of the factors for determining hybridization conditions is provided by Sambrook et al. ("Molecular

30   Cloning, a Laboratory Manual, 2nd ed., c. 1989 by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; *see esp.,* chapters 11 and 12). Additional considerations and details of the physical chemistry of hybridization are provided by G.H. Keller and M.M. Manak DNA Probes", 2nd Ed. pp. 1-25, c. 1993 by Stockton Press, New York, NY.

Depending on the stringency of the conditions under which these probes and/or primers are used, polynucleotides exhibiting a wide range of similarity to those in Table 1 can be detected or isolated. When the practitioner wishes to examine the result of membrane hybridizations under a variety of stringencies, an efficient way to do so is to perform the

5   hybridization under a low stringency condition, then to wash the hybridization membrane under increasingly stringent conditions.

When using SDFs to identify orthologous genes in other species, the practitioner will preferably adjust the amount of target DNA of each species so that, as nearly as is practical,

10   the same number of genome equivalents are present for each species examined. This prevents faint signals from species having large genomes, and thus small numbers of genome equivalents per mass of DNA, from erroneously being interpreted as absence of the corresponding gene in the genome.

The probes and/or primers of the instant invention can also be used to detect or isolate

15   nucleotides that are identical" to the probes or primers. Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below.

Isolated polynucleotides within the scope of the invention also include allelic variants of

20   the specific sequences presented in Table 1. The probes and/or primers of the invention can also be used to detect and/or isolate polynucleotides exhibiting at least 80% sequence identity with the sequences of Table 1 or fragments thereof.

With respect to nucleotide sequences, degeneracy of the genetic code provides the

25   possibility to substitute at least one base of the base sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the DNA of the present invention may also have any base sequence that has been changed from a sequence in Table 1 by substitution in accordance with degeneracy of genetic code. References describing codon usage include: Carels *et al.*, *J. Mol. Evol.* __46__: 45

30   (1998) and Fennoy *et al.*, *Nucl. Acids Res.* __21(23)__: 5294 (1993).

B.2. Mapping

The isolated SDF DNA of the invention can be used to create various types of genetic and physical maps of the genome of corn, Arabidopsis, soybean, rice, wheat, or other plants.

Some SDFs may be absolutely associated with particular phenotypic traits, allowing construction of gross genetic maps. While not all SDFs will immediately be associated with a phenotype, all SDFs can be used as probes for identifying polymorphisms associated with phenotypes of interest. Briefly, one method of mapping involves total DNA isolation from

5   individuals. It is subsequently cleaved with one or more restriction enzymes, separated according to mass, transferred to a solid support, hybridized with SDF DNA and the pattern of fragments compared. Polymorphisms associated with a particular SDF are visualized as differences in the size of fragments produced between individual DNA samples after digestion with a particular restriction enzyme and hybridization with the SDF. After

10   identification of polymorphic SDF sequences, linkage studies can be conducted. By using the individuals showing polymorphisms as parents in crossing programs, F2 progeny recombinants or recombinant inbreds, for example, are then analyzed. The order of DNA polymorphisms along the chromosomes can be determined based on the frequency with which they are inherited together versus independently. The closer two polymorphisms are

15   together in a chromosome the higher the probability that they are inherited together. Integration of the relative positions of all the polymorphisms and associated marker SDFs can produce a genetic map of the species, where the distances between markers reflect the recombination frequencies in that chromosome segment.

The use of recombinant inbred lines for such genetic mapping is described for

20   *Arabidopsis* by Alonso-Blanco et al. (*Methods in Molecular Biology*, vol.82, *Arabidopsis Protocols*", pp. 137-146, J.M. Martinez-Zapater and J. Salinas, eds., c. 1998 by Humana Press, Totowa, NJ) and for corn by Burr (Mapping Genes with Recombinant Inbreds", pp. 249-254. *In* Freeling, M. and V. Walbot (Ed.), *The Maize Handbook*, c. 1994 by Springer-Verlag New York, Inc.: New York, NY, USA; Berlin Germany; Burr et al. *Genetics* (1998)

25   118: 519; Gardiner, J. et al., (1993) *Genetics* 134: 917). This procedure, however, is not limited to plants and can be used for other organisms (such as yeast) or for individual cells.

The SDFs of the present invention can also be used for simple sequence repeat (SSR) mapping. Rice SSR mapping is described by Morgante et al. (*The Plant Journal* (1993) 3: 165), Panaud et al. (*Genome* (1995) 38: 1170); Senior et al. (*Crop Science* (1996) 36: 1676),

30   Taramino et al. (*Genome* (1996) 39: 277) and Ahn et al. (*Molecular and General Genetics* (1993) 241: 483-90). SSR mapping can be achieved using various methods. In one instance, polymorphisms are identified when sequence specific probes contained within an SDF flanking an SSR are made and used in polymerase chain reaction (PCR) assays with template DNA from two or more individuals of interest. Here, a change in the number of tandem

repeats between the SSR-flanking sequences produces differently sized fragments (U.S. Patent 5,766,847). Alternatively, polymorphisms can be identified by using the PCR fragment produced from the SSR-flanking sequence specific primer reaction as a probe against Southern blots representing different individuals (U.H. Refseth et al., (1997)

5   *Electrophoresis* 18: 1519).

Genetic and physical maps of crop species have many uses. For example, these maps can be used to devise positional cloning strategies for isolating novel genes from the mapped crop species. In addition, because the genomes of closely related species are largely syntenic (that is, they display the same ordering of genes within the genome), these maps can be used

10   to isolate novel alleles from relatives of crop species by positional cloning strategies.

The various types of maps discussed above can be used with the SDFs of the invention to identify Quantitative Trait Loci (QTLs). Many important crop traits, such as the solids content of tomatoes, are quantitative traits and result from the combined interactions of several genes. These genes reside at different loci in the genome, oftentimes on different

15   chromosomes, and generally exhibit multiple alleles at each locus. The SDFs of the invention can be used to identify QTLs and isolate specific alleles as described by de Vicente and Tanksley (*Genetics* 134:585 (1993)). In addition to isolating QTL alleles in present crop species, the SDFs of the invention can also be used to isolate alleles from the corresponding QTL of wild relatives. Transgenic plants having various combinations of QTL alleles can

20   then be created and the effects of the combinations measured. Once a desired allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (for review *see* Tanksley and McCouch, *Science* 277:1063 (1997)).

In another embodiment, the SDFs can be used to help create physical maps of the

25   genome of corn, *Arabidopsis* and related species. Where SDFs have been ordered on a genetic map, as described above, they can be used as probes to discover which clones in large libraries of plant DNA fragments in YACs, BACs, etc. contain the same SDF or similar sequences, thereby facilitating the assignment of the large DNA fragments to chromosomal positions. Subsequently, the large BACs, YACs, etc. can be ordered unambiguously by more

30   detailed studies of their sequence composition (e.g. Marra et al. (1997) Genomic Research 7:1072-1084) and by using their end or other sequences to find the identical sequences in other cloned DNA fragments. The overlapping of DNA sequences in this way allows large contigs of plant sequences to be built that, when sufficiently extended, provide a complete

physical map of a chromosome. Sometimes the SDFs themselves will provide the means of joining cloned sequences into a contig.

The patent publication WO95/35505 and U.S. Patents 5,445,943 and 5,410,270 describe scanning multiple alleles of a plurality of loci using hybridization to arrays of

5   oligonucleotides. These techniques are useful for each of the types of mapping discussed above.

Following the procedures described above and using a plurality of the SDFs of the present invention, any individual can be genotyped. These individual genotypes can be used for the identification of particular cultivars, varieties, lines, ecotypes and genetically

10   modified plants or can serve as tools for subsequent genetic studies involving multiple phenotypic traits.

B.3 Southern Blot Hybridization

The sequences from Table 1 can be used as probes for various hybridization

15   techniques. These techniques are useful for detecting target polynucleotides in a sample or for determining whether transgenic plants, seeds or host cells harbor a gene or sequence of interest and thus might be expected to exhibit a particular trait or phenotype.

In addition, the SDFs from the invention can be used to isolate additional members of gene families from the same or different species and/or orthologous genes from the same or

20   different species. This is accomplished by hybridizing an SDF to, for example, a Southern blot containing the appropriate genomic DNA or cDNA. Given the resulting hybridization data, one of ordinary skill in the art could distinguish and isolate the correct DNA fragments by size, restriction sites, sequence and stated hybridization conditions from a gel or from a library.

25   Identification and isolation of orthologous genes from closely related species and alleles within a species is particularly desirable because of their potential for crop improvement. Many important crop traits, such as the solid content of tomatoes, result from the combined interactions of the products of several genes residing at different loci in the genome. Generally, alleles at each of these loci can make quantitative differences to the trait.

30   By identifying and isolating numerous alleles for each locus from within or different species, transgenic plants with various combinations of alleles can be created and the effects of the combinations measured. Once a more favorable allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (Tanksley et al. *Science* 277:1063(1997)).

The results from hybridizations of the SDFs of the invention to, for example, Southern blots containing DNA from another species can also be used to generate restriction fragment maps for the corresponding genomic regions. These maps provide additional

5   information about the relative positions of restriction sites within fragments, further distinguishing mapped DNA from the remainder of the genome.

Physical maps can be made by digesting genomic DNA with different combinations of restriction enzymes.

Probes for Southern blotting to distinguish individual restriction fragments can range

10   in size from 15 to 20 nucleotides to several thousand nucleotides. More preferably, the probe is 100 to 1,000 nucleotides long for identifying members of a gene family when it is found that repetitive sequences would complicate the hybridization. For identifying an entire corresponding gene in another species, the probe is more preferably the length of the gene, typically 2,000 to 10,000 nucleotides, but probes 50-1,000 nucleotides long might be used.

15   Some genes, however, might require probes up to 1,500 nucleotides long or overlapping probes constituting the full-length sequence to span their lengths.

Also, while it is preferred that the probe be homogeneous with respect to its sequence, it is not necessary. For example, as described below, a probe representing members of a gene family having diverse sequences can be generated using PCR to amplify genomic DNA or

20   RNA templates using primers derived from SDFs that include sequences that define the gene family.

For identifying corresponding genes in another species, the next most preferable probe is a cDNA spanning the entire coding sequence, which allows all of the mRNA-coding fragment of the gene to be identified. Probes for Southern blotting can easily be generated

25   from SDFs by making primers having the sequence at the ends of the SDF and using corn or *Arabidopsis* genomic DNA as a template. In instances where the SDF includes sequence conserved among species, primers including the conserved sequence can be used for PCR with genomic DNA from a species of interest to obtain a probe.

Similarly, if the SDF includes a domain of interest, that fragment of the SDF can be used to

30   make primers and, with appropriate template DNA, used to make a probe to identify genes containing the domain. Alternatively, the PCR products can be resolved, for example by gel electrophoresis, and cloned and/or sequenced. Using Southern hybridization, the variants of the domain among members of a gene family, both within and across species, can be examined.

B.4.1 Isolating DNA from Related Organisms

The SDFs of the invention can be used to isolate the corresponding DNA from other organisms. Either cDNA or genomic DNA can be isolated. For isolating genomic DNA, a lambda, cosmid, BAC or YAC, or other large insert genomic library from the plant of interest can be constructed using standard molecular biology techniques as described in detail by Sambrook et al. 1989 (Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York) and by Ausubel et al. 1992 (Current Protocols in Molecular Biology, Greene Publishing, New York).

To screen a phage library, for example, recombinant lambda clones are plated out on appropriate bacterial medium using an appropriate *E. coli* host strain. The resulting plaques are lifted from the plates using nylon or nitrocellulose filters. The plaque lifts are processed through denaturation, neutralization, and washing treatments following the standard protocols outlined by Ausubel et al. (1992). The plaque lifts are hybridized to either radioactively labeled or non-radioactively labeled SDF DNA at room temperature for about 16 hours, usually in the presence of 50% formamide and 5X SSC (sodium chloride and sodium citrate) buffer and blocking reagents. The plaque lifts are then washed at 42°C with 1% Sodium Dodecyl Sulfate (SDS) and at a particular concentration of SSC. The SSC concentration used is dependent upon the stringency at which hybridization occurred in the initial Southern blot analysis performed. For example, if a fragment hybridized under medium stringency (e.g., Tm - 20°C), then this condition is maintained or preferably adjusted to a less stringent condition (e.g., Tm-30°C) to wash the plaque lifts. Positive clones show detectable hybridization e.g., by exposure to X-ray films or chromogen formation. The positive clones are then subsequently isolated for purification using the same general protocol outlined above. Once the clone is purified, restriction analysis can be conducted to narrow the region corresponding to the gene of interest. The restriction analysis and succeeding subcloning steps can be done using procedures described by, for example Sambrook et al. (1989) cited above.

The procedures outlined for the lambda library are essentially similar to those used for YAC library screening, except that the YAC clones are harbored in bacterial colonies. The YAC clones are plated out at reasonable density on nitrocellulose or nylon filters supported by appropriate bacterial medium in petri plates. Following the growth of the bacterial clones, the filters are processed through the denaturation, neutralization, and washing steps following

the procedures of Ausubel et al. 1992. The same hybridization procedures for lambda library screening are followed.

To isolate cDNA, similar procedures using appropriately modified vectors are employed. For instance, the library can be constructed in a lambda vector appropriate for cloning cDNA such as λgt11. Alternatively, the cDNA library can be made in a plasmid vector. cDNA for cloning can be prepared by any of the methods known in the art, but is preferably prepared as described above. Preferably, a cDNA library will include a high proportion of full-length clones.

B. 5. Isolating and/or Identifying Orthologous Genes

Probes and primers of the invention can be used to identify and/or isolate

5   polynucleotides related to those in Table 1. Related polynucleotides are those that are native to other plant organisms and exhibit either similar sequence or encode polypeptides with similar biological activity. One specific example is an orthologous gene. Orthologous genes have the same functional activity. As such, orthologous genes may be distinguished from homologous genes. The percentage of identity is a function of evolutionary separation and, in closely related

10   species, the percentage of identity can be 98 to 100%. The amino acid sequence of a protein encoded by an orthologous gene can be less than 75% identical, but tends to be at least75% or at least 80% identical, more preferably at least 90%, most preferably at least 95% identical to the amino acid sequence of the reference protein.

To find orthologous genes, the probes are hybridized to nucleic acids from a species of interest

15   under low stringency conditions, preferably one where sequences containing as much as 40-45% mismatches will be able to hybridize. This condition is established by T_m - 40°C to Tm - 48°C (*see* below). Blots are then washed under conditions of increasing stringency. It is preferable that the wash stringency be such that sequences that are 85 to 100% identical will hybridize. More preferably, sequences 90 to 100% identical will hybridize and most preferably only

20   sequences greater than 95% identical will hybridize. One of ordinary skill in the art will recognize that, due to degeneracy in the genetic code, amino acid sequences that are identical can be encoded by DNA sequences as little as 67% identical or less. Thus, it is preferable, for example, to make an overlapping series of shorter probes, on the order of 24 to 45 nucleotides, and individually hybridize them to the same arrayed library to avoid the problem of degeneracy

25   introducing large numbers of mismatches.

As evolutionary divergence increases, genome sequences also tend to diverge. Thus, one of skill will recognize that searches for orthologous genes between more divergent species will require the use of lower stringency conditions compared to searches between closely related species. Also, degeneracy of the genetic code is more of a problem for

5   searches in the genome of a species more distant evolutionarily from the species that is the source of the SDF probe sequences.

Therefore the method described in Bouckaert et al., U.S. Ser. No. 60/121,700 Atty. Dkt. No. 2750-117P, Client Dkt. No. 00010.001, filed February 25, 1999, hereby incorporated in its entirety by reference, can be applied to the SDFs of the present invention

10   to isolate related genes from plant species which do not hybridize to the corn *Arabidopsis*, soybean, rice, wheat, and other plant sequences of Table 1.

Identification of the relationship of nucleotide or amino acid sequences among plant species can be done by comparing the nucleotide or amino acid sequences of SDFs of the present application with nucleotide or amino acid sequences of other SDFs such as those

15   present in applications listed in the table below:

Country	Attorney No.	Client No.	Filed	Application No.
United States	2750-0301P	80002.001	9/4/98	60/099,672
United States	2750-0300P	80001.001	9/4/98	60/099,671
United States	2750-0302P	80003.001	9/11/98	60/099,933
United States	2750-0304P	80004.001	9/17/98	60/100,864
United States	2750-0305P	80005.001	9/18/98	60/101,042
United States	2750-0306P	80006.001	9/21/98	60/101,255
United States	2750-0307P	80007.001	9/24/98	60/101,682
United States	2750-0308P	80008.001	9/30/98	60/102,533
United States	2750-0309P	80009.001	9/30/98	60/102,460
United States	2750-0311P	80011.001	10/5/98	60/103,141
United States	2750-0310P	80010.001	10/5/98	60/103,116
United States	2750-0312P	80012.001	10/6/98	60/103,215
United States	2750-0313P	80013.001	10/8/98	60/103,554
United States	2750-0314P	80014.001	10/9/98	60/103,574
United States	2750-0315P	80015.001	10/13/98	60/103,907
United States	2750-0316P	80016.001	10/14/98	60/104,268
United States	2750-0317P	80017.001	10/16/98	60/104,680
United States	2750-0318P	80018.001	10/19/98	60/104,828
United States	2750-0319P	80019.001	10/20/98	60/105,008
United States	2750-0320P	80020.001	10/21/98	60/105,142
United States	2750-0321P	80021.001	10/22/98	60/105,533
United States	2750-0322P	80022.001	10/26/98	60/105,571
United States	2750-0323P	80023.001	10/27/98	60/105,815
United States	2750-0324P	80024.001	10/29/98	60/106,105
United States	2750-0325P	80025.001	10/30/98	60/106,218
United States	2750-0326P	80026.001	11/2/98	60/106,685
United States	2750-0327P	80027.001	11/6/98	60/107,282

Country	Attorney No.	Client No.	Filed	Application No.
United States	2750-0329P	80029.001	11/9/98	60/107,719
United States	2750-0328P	80028.001	11/9/98	60/107,720
United States	2750-0330P	80030.001	11/10/98	60/107,836
United States	2750-0331P	80031.001	11/12/98	60/108,190
United States	2750-0332P	80032.001	11/16/98	60/108,526
United States	2750-0333P	80033.001	11/17/98	60/108,901
United States	2750-0334P	80034.001	11/19/98	60/109,124
United States	2750-0335P	80035.001	11/19/98	60/109,127
United States	2750-0336P	80036.001	11/20/98	60/109,267
United States	2750-0337P	80037.001	11/23/98	60/109,594
United States	2750-0339P	80039.001	11/25/98	60/110,050
United States	2750-0338P	80038.001	11/25/98	60/110,053
United States	2750-0340P	80040.001	11/27/98	60/110,158
United States	2750-0341P	80041.001	11/30/98	60/110,263
United States	2750-0342P	80042.001	12/1/98	60/110,495
United States	2750-0343P	80043.001	12/2/98	60/110,626
United States	2750-0344P	80044.001	12/3/98	60/110,701
United States	2750-0345P	80045.001	12/7/98	60/111,339
United States	2750-0346P	80046.001	12/9/98	60/111,589
United States	2750-0347P	80047.001	12/10/98	60/111,782
United States	2750-0348P	80048.001	12/11/98	60/111,812
United States	2750-0349P	80049.001	12/14/98	60/112,096
United States	2750-0350P	80050.001	12/15/98	60/112,224
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United States	2750-0352P	80052.001	12/17/98	60/112,862
United States	2750-0353P	80053.001	12/18/98	60/112,912
United States	2750-0354P	80054.001	12/21/98	60/113,248
United States	2750-0355P	80055.001	12/22/98	60/113,522
United States	2750-0356P	80056.001	12/23/98	60/113,826
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United States	2750-0359P	80059.001	12/30/98	60/114,455
United States	2750-0360P	80060.001	1/4/99	60/114,740
United States	2750-0361P	80061.001	1/6/99	60/114,866
United States	2750-0367P	80067.001	1/7/99	60/115,154
United States	2750-0364P	80064.001	1/7/99	60/115,151
United States	2750-0363P	80063.001	1/7/99	60/115,152
United States	2750-0362P	80062.001	1/7/99	60/115,153
United States	2750-0366P	80066.001	1/7/99	60/115,156
United States	2750-0365P	80065.001	1/7/99	60/115,155
United States	2750-0370P	80070.001	1/8/99	60/115,293
United States	2750-0369P	80069.001	1/8/99	60/115,365
United States	2750-0368P	80068.001	1/8/99	60/115,364
United States	2750-0371P	80071.001	1/11/99	60/115,339
United States	2750-0372P	80072.001	1/12/99	60/115,518
United States	2750-0373P	80073.001	1/13/99	60/115,847
United States	2750-0374P	80074.001	1/14/99	60/115,905
United States	2750-0376P	80076.001	1/15/99	60/116,384
United States	2750-0375P	80075.001	1/15/99	60/116,383
United States	2750-0377P	80077.001	1/19/99	60/116,329

Country	Attorney No.	Client No.	Filed	Application No.
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United States	2750-0380P	80080.001	1/21/99	60/116,672
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United States	2750-0381P	80081.001	1/22/99	60/116,960
United States	2750-0383P	80083.001	1/28/99	60/117,756
United States	2750-0384P	80084.001	2/3/99	60/118,672
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United States	2750-0386P	80086.001	2/5/99	60/118,778
United States	2750-0387P	80087.001	2/8/99	60/119,029
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United States	2750-0392P	80092.001	2/16/99	60/120,196
United States	2750-0394P	80094.001	2/18/99	60/120,583
United States	2750-0395P	80095.001	2/22/99	60/121,072
United States	2750-0396P	80096.001	2/23/99	60/121,334
United States	2750-0397P	80097.001	2/24/99	60/121,470
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United States	2750-0398P	80098.001	2/25/99	60/121,704
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United States	2750-0425P	80125.001	4/30/99	60/132,048
United States	2750-0426P	80126.001	5/4/99	60/132,484
United States	2750-0427P	80127.001	5/5/99	60/132,485

Country	Attorney No.	Client No.	Filed	Application No.
United States	2750-0428P	91002.001	5/6/99	60/132,487
United States	2750-0429P	80129.001	5/6/99	60/132,486
United States	2750-0430P	80130.001	5/7/99	60/132,863
United States	2750-0431P	80131.001	5/11/99	60/134,256
United States	2750-0433P	00025.001	5/14/99	60/134,221
United States	2750-0432P	91006.001	5/14/99	60/134,370
United States	2750-0434P	80116.001	5/14/99	60/134,219
United States	2750-0435P	80117.001	5/14/99	60/134,218
United States	2750-0436P	91007.001	5/18/99	60/134,768
United States	2750-0437P	91008.001	5/19/99	60/134,941
United States	2750-0438P	91009.001	5/20/99	60/135,124
United States	2750-0439P	91010.001	5/21/99	60/135,353
United States	2750-0440P	91011.001	5/24/99	60/135,629
United States	2750-0441P	91012.001	5/25/99	60/136,021
United States	2750-0442P	91013.001	5/27/99	60/136,392
United States	2750-0444P	91014.001	5/28/99	60/136,782
United States	2750-0445P	91015.001	6/1/99	60/137,222
United States	2750-0446P	91016.001	6/3/99	60/137,528
United States	2750-0447P	91017.001	6/4/99	60/137,502
United States	2750-0449P	91018.001	6/7/99	60/137,724
United States	2750-0450P	91019.001	6/8/99	60/138,094
United States	2750-0458P	00033.002	6/10/99	60/138,847
United States	2750-0457P	00033.001	6/10/99	60/138,540
United States	2750-0463P	00034.001	6/14/99	60/139,119
United States	2750-0462P	80132.012	6/16/99	60/139,452
United States	2750-0461P	80132.011	6/16/99	60/139,453
United States	2750-0464P	00037.001	6/17/99	60/139,492
United States	2750-0454P	80132.006	6/18/99	60/139,457
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United States	2750-0466P	00039.001	6/18/99	60/139,750
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United States	2750-0455P	80132.007	6/18/99	60/139,460
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United States	2750-0452P	80132.004	6/18/99	60/139,461
United States	2750-0451P	80132.003	6/18/99	60/139,459
United States	2750-0448P	80132.002	6/18/99	60/139,454
United States	2750-0443P	80132.001	6/18/99	60/139,458
United States	2750-0456P	80132.008	6/18/99	60/139,456
United States	2750-0467P	00042.001	6/21/99	60/139,817
United States	2750-0468P	00043.001	6/22/99	60/139,899
United States	2750-0469P	00044.001	6/23/99	60/140,354
United States	2750-0470P	00042.002	6/23/99	60/140,353
United States	2750-0471P	00045.001	6/24/99	60/140,695
United States	2750-0472P	00046.001	6/28/99	60/140,823
United States	2750-0473P	00048.001	6/29/99	60/140,991
United States	2750-0474P	00049.001	6/30/99	60/141,287
United States	2750-0475P	00050.001	7/1/99	60/141,842
United States	2750-0476P	00051.001	7/1/99	60/142,154
United States	2750-0477P	00052.001	7/2/99	60/142,055

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Country	Attorney No.	Client No.	Filed	Application No.
United States	2750-0478P	00053.001	7/6/99	60/142,390
United States	2750-0479P	00054.001	7/8/99	60/142,803
United States	2750-0480P	00058.001	7/9/99	60/142,920
United States	2750-0481P	00059.001	7/12/99	60/142,977
United States	2750-0482P	00060.001	7/13/99	60/143,542
United States	2750-0489P	00061.001	7/14/99	60/143,624
United States	2750-0490P	00062.001	7/15/99	60/144,005
United States	2750-0486P	80134.004	7/16/99	60/144,085
United States	2750-0485P	80134.003	7/16/99	60/144,086
United States	2750-0494P	80134.010	7/19/99	60/144,333
United States	2750-0497P	00064.001	7/19/99	60/144,325
United States	2750-0488P	80134.006	7/19/99	60/144,332
United States	2750-0495P	80134.013	7/19/99	60/144,335
United States	2750-0492P	80134.008	7/19/99	60/144,331
United States	2750-0496P	80134.014	7/19/99	60/144,334
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United States	2750-0483P	80134.001	7/21/99	60/145,088
United States	2750-0484P	80134.002	7/21/99	60/145,086
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United States	2750-0527P	80141.003	8/12/99	60/148,340
United States	2750-0528P	80141.004	8/12/99	60/148,337

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United States	2750-0525P	80141.001	8/12/99	60/148,347
United States	2750-0529P	00083.001	8/13/99	60/148,565
United States	2750-0532P	80142.002	8/13/99	60/148,684
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United States	2750-0534P	80001.003	8/17/99	60/149,928
United States	2750-0537P	00084.001	8/17/99	60/149,175
United States	2750-0536P	80001.005	8/17/99	60/149,925
United States	2750-0535P	80001.004	8/17/99	60/149,926
United States	2750-0533P	80001.002	8/17/99	60/149,927
United States	2750-0538P	00085.001	8/18/99	60/149,426
United States	2750-0539P	00086.001	8/20/99	60/149,722
United States	2750-0541P	80143.002	8/20/99	60/149,929
United States	2750-0542P	00087.001	8/20/99	60/149,723
United States	2750-0540P	80143.001	8/23/99	60/149,930
United States	2750-0543P	00088.001	8/23/99	60/149,902
United States	2750-0544P	00089.001	8/25/99	60/150,566
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United States	2750-0548P	00091.001	8/27/99	60/151,080
United States	2750-0546P	80144.002	8/27/99	60/151,066
United States	2750-0545P	80144.001	8/27/99	60/151,065
United States	2750-0549P	00092.001	8/30/99	60/151,303
United States	2750-0552P	00093.001	8/31/99	60/151,438
United States	2750-0553P	00094.001	9/1/99	60/151,930
United States	2750-0550P	80001.006	9/3/99	09/391,631
International	2750-0551F(PC)	80001.100	9/3/99	99/204,38
United States	2750-0554P	00095.001	9/7/99	60/152,363
United States	2750-0555P	00096.001	9/10/99	60/153,070
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United States	2750-0558P	00101.001	9/16/99	60/154,039
United States	2750-0559P	00102.001	9/20/99	60/154,779
United States	2750-0560P	00103.001	9/22/99	60/155,139
United States	2750-0561P	00104.001	9/23/99	60/155,486
United States	2750-0562P	00105.001	9/24/99	60/155,659
United States	2750-0563P	00106.001	9/28/99	60/156,458
United States	2750-0564P	00107.001	9/29/99	60/156,596
United States	2750-0570P	00108.001	10/4/99	60/157,117
United States	2750-0571P	00109.001	10/5/99	60/157,753
International	2750-0568F(PC)	80010.101	10/5/99	99/228,54
International	2750-0569F(PC)	80010.102	10/5/99	99/228,53
United States	2750-0565P	80010.002	10/5/99	09/413,198
United States	2750-0566P	80010.003	10/5/99	09/412,922
International	2750-0567F(PC)	80010.100	10/5/99	99/228,55
United States	2750-0572P	00110.001	10/6/99	60/157,865
United States	2750-0575P	00111.001	10/7/99	60/158,029
United States	2750-0576P	00112.001	10/8/99	60/158,232
United States	2750-0577P	00113.001	10/12/99	60/158,369
United States	2750-0583P	80148.002	10/13/99	60/159,294
United States	2750-0579P	80146.002	10/13/99	60/159,293

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United States	2750-0574P	80145.002	10/13/99	60/159,295
United States	2750-0573P	80145.001	10/14/99	60/159,330
United States	2750-0580P	80147.001	10/14/99	60/159,638
United States	2750-0581P	80147.002	10/14/99	60/159,637
United States	2750-0582P	80148.001	10/14/99	60/159,329
United States	2750-0578P	80146.001	10/14/99	60/159,331
United States	2750-0584P	00116.001	10/18/99	60/159,584
United States	2750-0590P	80150.002	10/21/99	60/160,767
United States	2750-0589P	80150.001	10/21/99	60/160,768
United States	2750-0588P	00119.001	10/21/99	60/160,741
United States	2750-0587P	80149.002	10/21/99	60/160,770
United States	2750-0586P	80149.001	10/21/99	60/160,814
United States	2750-0585P	00118.001	10/21/99	60/160,815
United States	2750-0591P	00120.001	10/22/99	60/160,980
United States	2750-0592P	80151.001	10/22/99	60/160,989
United States	2750-0593P	80151.002	10/22/99	60/160,981
United States	2750-0594P	00121.001	10/25/99	60/161,405
United States	2750-0595P	80152.001	10/25/99	60/161,406
United States	2750-0596P	80152.002	10/25/99	60/161,404
United States	2750-0599P	80153.002	10/26/99	60/161,359
United States	2750-0597P	00122.001	10/26/99	60/161,361
United States	2750-0598P	80153.001	10/26/99	60/161,360
United States	2750-0603P	80154.002	10/28/99	60/161,993
United States	2750-0602P	80154.001	10/28/99	60/161,992
United States	2750-0601P	00123.001	10/28/99	60/161,920
United States	2750-0600P	80026.002	10/28/99	09/428,944
United States	2750-0605P	80155.001	10/29/99	60/162,142
United States	2750-0606P	80155.002	10/29/99	60/162,228
United States	2750-0604P	00124.001	10/29/99	60/162,143
United States	2750-0607P	00125.001	11/1/99	60/162,894
United States	2750-0608P	80156.001	11/1/99	60/162,891
United States	2750-0609P	80156.002	11/1/99	60/162,895
United States	2750-0610P	00126.001	11/2/99	60/163,093
United States	2750-0611P	80157.001	11/2/99	60/163,092
United States	2750-0612P	80157.002	11/2/99	60/163,091
United States	2750-0615P	80158.002	11/3/99	60/163,281
United States	2750-0613P	00127.001	11/3/99	60/163,249
United States	2750-0614P	80158.001	11/3/99	60/163,248
United States	2750-0616P	00128.001	11/4/99	60/163,379
United States	2750-0617P	80159.001	11/4/99	60/163,381
United States	2750-0618P	80159.002	11/4/99	60/163,380
United States	2750-0620P	80160.001	11/8/99	60/164,151
United States	2750-0619P	00129.001	11/8/99	60/164,146
United States	2750-0621P	80160.002	11/8/99	60/164,150
United States	2750-0625P	80162.002	11/9/99	60/164,259
United States	2750-0623P	80161.002	11/9/99	60/164,260
United States	2750-0628P	00131.001	11/10/99	60/164,544
United States	2750-0629P	80164.001	11/10/99	60/164,545
United States	2750-0627P	80163.002	11/10/99	60/164,318
United States	2750-0626P	80163.001	11/10/99	60/164,321

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United States	2750-0622P	80161.001	11/10/99	60/164,319
United States	2750-0630P	80164.002	11/10/99	60/164,548
United States	2750-0624P	80162.001	11/10/99	60/164,317
United States	2750-0631P	00132.001	11/12/99	60/164,961
United States	2750-0636P	80166.002	11/12/99	60/164,962
United States	2750-0635P	80166.001	11/12/99	60/164,959
United States	2750-0634P	00133.001	11/12/99	60/164,870
United States	2750-0632P	80165.001	11/12/99	60/164,871
United States	2750-0633P	80165.002	11/12/99	60/164,960
United States	2750-0637P	00134.001	11/15/99	60/164,927
United States	2750-0638P	80167.001	11/15/99	60/164,929
United States	2750-0639P	80167.002	11/15/99	60/164,926
United States	2750-0642P	80168.002	11/16/99	60/165,661
United States	2750-0640P	00135.001	11/16/99	60/165,669
United States	2750-0641P	80168.001	11/16/99	60/165,671
United States	2750-0643P	00136.001	11/17/99	60/165,919
United States	2750-0644P	80169.001	11/17/99	60/165,918
United States	2750-0645P	80169.002	11/17/99	60/165,911
United States	2750-0646P	00137.001	11/18/99	60/166,157
United States	2750-0647P	80170.001	11/18/99	60/166,173
United States	2750-0648P	80170.002	11/18/99	60/166,158
United States	2750-0650P	80171.001	11/19/99	60/166,411
United States	2750-0651P	80171.002	11/19/99	60/166,412
United States	2750-0649P	00139.001	11/19/99	60/166,419
United States	2750-0653P	80172.001	11/22/99	60/166,750
United States	2750-0652P	00140.001	11/22/99	60/166,733
United States	2750-0655P	80173.002	11/23/99	60/167,362
United States	2750-0658P	80174.002	11/24/99	60/167,235
United States	2750-0654P	80173.001	11/24/99	60/167,382
United States	2750-0656P	00141.001	11/24/99	60/167,233
United States	2750-0657P	80174.001	11/24/99	60/167,234
United States	2750-0659P	00142.001	11/30/99	60/167,904
United States	2750-0660P	80175.001	11/30/99	60/167,908
United States	2750-0661P	80175.002	11/30/99	60/167,902
United States	2750-0665P	80176.002	12/1/99	60/168,231
United States	2750-0662P	80042.002	12/1/99	09/451,320
United States	2750-0664P	80176.001	12/1/99	60/168,233
United States	2750-0663P	00143.001	12/1/99	60/168,232
United States	2750-0668P	80177.002	12/2/99	60/168,548
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United States	2750-0670P	80178.001	12/3/99	60/168,673
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United States	2750-0672P	00147.001	12/7/99	60/169,298
United States	2750-0673P	80179.001	12/7/99	60/169,278
United States	2750-0674P	80179.002	12/7/99	60/169,302
United States	2750-0676P	80180.002	12/8/99	60/169,691
United States	2750-0675P	80180.001	12/8/99	60/169,692
United States	2750-0678P	80181.001	12/16/99	60/171,114

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United States	2750-0679P	80181.002	12/16/99	60/171,098
United States	2750-0677P	00149.001	12/16/99	60/171,107
United States	2750-0683P	80060.002	1/4/00	09/478,081
United States	2750-0684P	80070.002	1/7/00	09/479,221
International	2750-0686F(PC)	80070.100	1/7/00	00/004,66
United States	2750-0688P	80184.002	1/19/00	60/176,910
United States	2750-0681P	80182.002	1/19/00	60/176,866
United States	2750-0685P	80183.002	1/19/00	60/176,867
United States	2750-0689P	00152.001	1/26/00	60/178,166
United States	2750-0691P	80185.001	1/27/00	60/177,666
United States	2750-0687P	80184.001	1/27/00	60/178,545
United States	2750-0682P	80183.001	1/27/00	60/178,546
United States	2750-0680P	80182.001	1/27/00	60/178,544
United States	2750-0690P	00153.001	1/27/00	60/178,547
United States	2750-0692P	00155.001	1/28/00	60/178,754
United States	2750-0693P	80186.001	1/28/00	60/178,755
United States	2750-0695P	00157.001	2/1/00	60/179,395
United States	2750-0696P	80187.001	2/1/00	60/179,388
United States	2750-0697P	00158.001	2/3/00	60/180,039
United States	2750-0698P	80188.001	2/3/00	60/180,139
United States	2750-0694P	80084.002	2/3/00	09/497,191
United States	2750-0700P	80189.001	2/4/00	60/180,207
United States	2750-0699P	00159.001	2/4/00	60/180,206
United States	2750-0701P	00160.001	2/7/00	60/180,695
United States	2750-0702P	80190.001	2/7/00	60/180,696
United States	2750-0703P	00161.001	2/9/00	60/181,228
United States	2750-0704P	80191.001	2/9/00	60/181,214
United States	2750-0705P	00162.001	2/10/00	60/181,476
United States	2750-0706P	80192.001	2/10/00	60/181,551
United States	2750-0707P	00163.001	2/15/00	60/182,477
United States	2750-0713P	80194.001	2/15/00	60/182,478
United States	2750-0708P	80193.001	2/15/00	60/182,516
United States	2750-0712P	00164.001	2/15/00	60/182,512
United States	2750-0714P	00165.001	2/17/00	60/183,166
United States	2750-0715P	80195.001	2/17/00	60/183,165
United States	2750-0717P	80196.001	2/24/00	60/184,658
United States	2750-0716P	00167.001	2/24/00	60/184,667
Europe	2750-0709F(EP)	80090.103	2/25/00	00/301,439
Mexico	2750-0709F(MX)	80090.101	2/25/00	00/001,973
Canada	2750-0709F(CA)	80090.102	2/25/00	23/006,92
United States	2750-0719P	00168.001	2/25/00	60/185,118
United States	2750-0718P	91022.001	2/25/00	60/185,140
United States	2750-0709P	80090.002	2/25/00	09/513,996
United States	2750-0720P	80197.001	2/25/00	60/185,119
United States	2750-0721P	91023.001	2/28/00	60/185,398
United States	2750-0722P	00169.001	2/28/00	60/185,396
United States	2750-0723P	80198.001	2/28/00	60/185,397
United States	2750-0724P	91024.001	2/29/00	60/185,750
United States	2750-0710P	80100.002	3/1/00	09/517,537
United States	2750-0725P	00170.001	3/1/00	

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United States	2750-0726P	80199.001	3/1/00	60/186,296
United States	2750-0727P	91025.001	3/1/00	60/186,277
United States	2750-0711P	00171.001	3/2/00	60/186,390
United States	2750-0728P	80200.001	3/2/00	60/187,178
United States	2750-0729P	00172.001	3/2/00	60/186,386
United States	2750-0730P	80201.001	3/2/00	60/186,387
United States	2750-0731P	91026.001	3/3/00	60/186,670
United States	2750-0732P	00173.001	3/3/00	60/186,748
United States	2750-0733P	80202.001	3/3/00	60/186,669
United States	2750-0735P	91027.001	3/7/00	60/187,379
United States	2750-0734P	00174.001	3/7/00	60/187,378
United States	2750-0736P	00175.001	3/8/00	60/187,896
United States	2750-0737P	80203.001	3/8/00	60/187,888
United States	2750-0738P	91028.001	3/9/00	60/187,985
United States	2750-0739P	00177.001	3/10/00	60/188,187
United States	2750-0743P	80205.001	3/10/00	60/188,175
United States	2750-0742P	00178.001	3/10/00	60/188,185
United States	2750-0740P	80204.001	3/10/00	60/188,186
United States	2750-0741P	91030.001	3/10/00	60/188,174
United States	2750-0744P	91031.001	3/13/00	60/188,687
United States	2750-0745P	00179.001	3/14/00	60/189,080
United States	2750-0746P	80206.001	3/14/00	60/189,052
United States	2750-0747P	91032.001	3/15/00	60/189,460
United States	2750-0748P	00180.001	3/15/00	60/189,461
United States	2750-0749P	80207.001	3/15/00	60/189,462
United States	2750-0753P	80211.001	3/16/00	60/190,121
United States	2750-0756P	80212.001	3/16/00	60/189,959
United States	2750-0757P	91034.001	3/16/00	60/189,965
United States	2750-0750P	80208.001	3/16/00	60/190,120
United States	2750-0752P	80210.001	3/16/00	60/189,948
United States	2750-0754P	91033.001	3/16/00	60/189,958
United States	2750-0751P	80209.001	3/16/00	60/189,947
United States	2750-0755P	00181.001	3/16/00	60/189,953
United States	2750-0759P	80213.001	3/20/00	60/190,070
United States	2750-0762P	80214.001	3/20/00	60/190,089
United States	2750-0761P	00183.001	3/20/00	60/190,545
United States	2750-0758P	00182.001	3/20/00	60/190,069
United States	2750-0760P	91035.001	3/20/00	60/190,060
United States	2750-0764P	80215.001	3/22/00	60/191,097
United States	2750-0763P	00184.001	3/22/00	60/191,084
United States	2750-0767P	80216.001	3/23/00	60/191,545
United States	2750-0766P	00185.001	3/23/00	60/191,543
United States	2750-0765P	91036.001	3/23/00	60/191,549
United States	2750-0769P	00186.001	3/24/00	60/191,823
United States	2750-0770P	80217.001	3/24/00	60/191,825
United States	2750-0768P	91037.001	3/24/00	60/191,826
United States	2750-0772P	00187.001	3/27/00	60/192,421
United States	2750-0773P	80218.001	3/27/00	60/192,308
United States	2750-0771P	91038.001	3/27/00	60/192,420
United States	2750-0774P	91039.001	3/29/00	60/192,855

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United States	2750-0775P	00188.001	3/29/00	60/192,940
United States	2750-0776P	80219.001	3/29/00	60/192,941
United States	2750-0777P	91040.001	3/30/00	60/193,243
United States	2750-0778P	00189.001	3/30/00	60/193,244
United States	2750-0779P	80220.001	3/30/00	60/193,245
United States	2750-0782P	80221.001	3/31/00	60/193,455
United States	2750-0780P	91041.001	3/31/00	60/193,469
United States	2750-0781P	00190.001	3/31/00	60/193,453
United States	2750-0785P	91042.001	4/4/00	60/194,385
United States	2750-0787P	80222.001	4/4/00	60/194,398
United States	2750-0786P	00191.001	4/4/00	60/194,404
United States	2750-0792P	91044.001	4/5/00	60/194,698
United States	2750-0791P	80223.001	4/5/00	60/194,697
United States	2750-0789P	91043.001	4/5/00	60/194,682
United States	2750-0790P	00192.001	4/5/00	60/194,683
United States	2750-0793P	00193.001	4/6/00	60/194,874
Canada	2750-0783F(CA)	91000.102	4/6/00	02/302,828
Europe	2750-0783F(EP)	91000.101	4/6/00	00/302,919
United States	2750-0796P	80225.001	4/6/00	60/195,045
United States	2750-0783P	91000.002	4/6/00	09/543,680
Mexico	2750-0783F(MX)	91000.100	4/6/00	00/003,391
United States	2750-0784P	91045.001	4/6/00	60/194,884
United States	2750-0795P	00194.001	4/6/00	60/194,885
United States	2750-0794P	80224.001	4/6/00	60/194,872
United States	2750-0799P	80226.001	4/7/00	60/195,257
United States	2750-0797P	91046.001	4/7/00	60/195,258
United States	2750-0798P	00195.001	4/7/00	60/195,283
United States	2750-0804P	80228.001	4/11/00	60/196,089
United States	2750-0801P	80227.002	4/11/00	60/196,211
United States	2750-0802P	91047.001	4/11/00	60/196,168
United States	2750-0803P	00196.001	4/11/00	60/196,169
United States	2750-0800P	80227.001	4/12/00	60/196,212
United States	2750-0805P	91048.001	4/12/00	60/196,483
United States	2750-0806P	00197.001	4/12/00	60/196,487
United States	2750-0807P	80229.001	4/12/00	60/196,289
United States	2750-0809P	80230.001	4/12/00	60/196,486
United States	2750-0808P	00200.001	4/12/00	60/196,485
United States	2750-0811P	80231.002	4/13/00	60/196,213
United States	2750-0810P	80231.001	4/14/00	
United States	2750-0814P	91049.001	4/14/00	60/197,397
United States	2750-0816P	80233.001	4/17/00	60/197,678
United States	2750-0819P	80234.001	4/17/00	60/197,671
United States	2750-0812P	80232.001	4/17/00	60/197,870
United States	2750-0813P	80232.002	4/17/00	60/197,871
United States	2750-0817P	91050.001	4/17/00	60/198,268
United States	2750-0815P	00201.001	4/17/00	60/197,687
United States	2750-0818P	00202.001	4/17/00	60/198,133
United States	2750-0822P	80235.001	4/19/00	60/198,373
United States	2750-0821P	00203.001	4/19/00	60/198,386
United States	2750-0820P	91051.001	4/19/00	60/198,400

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United States	2750-0825P	80236.001	4/20/00	60/198,623
United States	2750-0824P	00204.001	4/20/00	60/198,619
United States	2750-0823P	91052.001	4/20/00	60/198,629
United States	2750-0826P	91053.001	4/21/00	60/198,765
United States	2750-0828P	80237.001	4/21/00	60/198,763
United States	2750-0827P	00206.001	4/21/00	60/198,767
United States	2750-0829P	91054.001	4/24/00	60/199,123
United States	2750-0830P	00207.001	4/24/00	60/199,124
United States	2750-0831P	80238.001	4/24/00	60/199,122
United States	2750-0832P	92001.001	4/26/00	60/200,034
United States	2750-0834P	00208.001	4/26/00	60/199,828
United States	2750-0835P	80239.001	4/26/00	60/199,818
United States	2750-0833P	92002.001	4/26/00	60/200,031
United States	2750-0836P	00210.001	4/27/00	60/200,103
United States	2750-0837P	80240.001	4/27/00	60/200,102
United States	2750-0788P	80123.002	4/28/00	09/559,232
United States	2750-0846P	80243.002	4/28/00	60/200,773
United States	2750-0844P	80242.002	4/28/00	60/200,373
United States	2750-0841P	92001.002	5/1/00	60/201,017
United States	2750-0842P	92002.002	5/1/00	60/201,018
United States	2750-0843P	80242.001	5/1/00	60/200,763
United States	2750-0847P	80244.001	5/1/00	60/200,762
United States	2750-0845P	80243.001	5/1/00	60/201,016
United States	2750-0839P	80241.001	5/1/00	60/200,879
United States	2750-0840P	91055.001	5/1/00	60/200,885
United States	2750-0848P	80244.002	5/1/00	60/200,761
United States	2750-0849P	91056.001	5/2/00	60/201,279
United States	2750-0850P	80245.001	5/2/00	60/201,305
United States	2750-0838P	00211.001	5/2/00	60/201,275
Mexico	2750-0851F(MX)	91002.102	5/4/00	00/004,406
United States	2750-0857P	00212.001	5/4/00	60/201,740
United States	2750-0858P	80246.001	5/4/00	60/201,750
United States	2750-0856P	91057.001	5/4/00	60/201,751
United States	2750-0852P	80126.002	5/4/00	09/566,262
United States	2750-0859P	91058.001	5/5/00	60/202,178
Europe	2750-0851F(EP)	91002.101	5/5/00	00/303,770
Canada	2750-0851F(CA)	91002.100	5/5/00	02/305,695
United States	2750-0860P	00213.001	5/5/00	60/202,112
United States	2750-0851P	91002.002	5/5/00	09/565,308
United States	2750-0861P	80247.001	5/5/00	60/202,180
United States	2750-0855P	80130.002	5/5/00	09/565,310
United States	2750-0854P	80129.002	5/5/00	09/565,307
United States	2750-0853P	80127.002	5/5/00	09/565,309
United States	2750-0865P	00215.001	5/9/00	60/202,919
United States	2750-0863P	80248.001	5/9/00	60/202,636
United States	2750-0864P	91059.001	5/9/00	60/202,915
United States	2750-0866P	80249.001	5/9/00	60/202,634
United States	2750-0862P	00214.001	5/9/00	60/202,914
United States	2750-0877P	91060.001	5/10/00	60/202,969
United States	2750-0879P	80252.001	5/10/00	60/202,963

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United States	2750-0878P	00216.001	5/10/00	60/202,968
United States	2750-0870P	80251.002	5/11/00	60/203,622
United States	2750-0869P	80251.001	5/11/00	60/203,669
United States	2750-0871P	80131.002	5/11/00	09/572,408
United States	2750-0881P	00217.001	5/11/00	60/203,457
United States	2750-0880P	91061.001	5/11/00	60/203,458
United States	2750-0868P	80250.002	5/11/00	60/203,672
United States	2750-0867P	80250.001	5/11/00	60/203,671
United States	2750-0882P	80253.001	5/11/00	60/203,279
Mexico	2750-0875F(MX)	91006.102	5/12/00	00/004,628
United States	2750-0873P	00025.002	5/12/00	09/570,582
Europe	2750-0875F(EP)	91006.101	5/12/00	00/304,017
United States	2750-0874P	80116.002	5/12/00	09/570,738
United States	2750-0875P	91006.002	5/12/00	09/570,581
United States	2750-0883P	91062.001	5/12/00	60/203,911
United States	2750-0884P	00219.001	5/12/00	60/203,916
United States	2750-0885P	80254.001	5/12/00	60/203,915
Canada	2750-0875F(CA)	91006.100	5/12/00	02/306,232
United States	2750-0872P	80117.002	5/12/00	09/570,768
United States	2750-0886P	91063.001	5/15/00	60/204,395
United States	2750-0888P	80255.001	5/15/00	60/204,122
United States	2750-0887P	00220.001	5/15/00	60/204,388
United States	2750-0891P	00221.001	5/16/00	60/204,568
United States	2750-0892P	80256.001	5/16/00	60/204,569
United States	2750-0894P	80257.001	5/17/00	60/204,829
United States	2750-0893P	00222.001	5/17/00	60/204,830
United States	2750-0890P	92002.003	5/17/00	60/205,325
United States	2750-0889P	92001.003	5/17/00	60/205,233
Canada	2750-0876F(CA)	91007.100	5/18/00	02/306,202
United States	2750-0896P	80258.001	5/18/00	60/205,058
Europe	2750-0876F(EP)	91007.101	5/18/00	00/304,161
United States	2750-0876P	91007.002	5/18/00	09/573,655
Mexico	2750-0876F(MX)	91007.102	5/18/00	00/004,850
United States	2750-0895P	00223.001	5/18/00	60/205,201
United States	2750-0898P	80259.001	5/19/00	60/205,243
United States	2750-0897P	00224.001	5/19/00	60/205,242
United States	2750-0900P	00225.001	5/22/00	60/205,572
United States	2750-0899P	91064.001	5/22/00	60/205,574
United States	2750-0901P	80260.001	5/22/00	60/205,576
United States	2750-0903P	80261.001	5/23/00	60/206,319
United States	2750-0902P	00226.001	5/23/00	60/206,316
United States	2750-0905P	80262.001	5/24/00	60/206,545
United States	2750-0904P	00227.001	5/24/00	60/206,553
United States	2750-0906P	91065.001	5/25/00	60/206,988
United States	2750-0908P	80263.001	5/26/00	60/207,243
United States	2750-0910P	00229.001	5/26/00	60/207,239
United States	2750-0911P	80264.001	5/26/00	60/207,354
United States	2750-0907P	00228.001	5/26/00	60/207,367
United States	2750-0909P	91066.001	5/26/00	60/207,242
United States	2750-0913P	00230.001	5/30/00	60/207,452

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United States	2750-0912P	91067.001	5/30/00	60/207,291
United States	2750-0914P	80265.001	5/30/00	60/207,329
United States	2750-0916P	80266.002	6/1/00	60/208,421
United States	2750-0918P	80267.002	6/1/00	60/208,648
United States	2750-0919P	91068.001	6/1/00	60/208,324
United States	2750-0920P	00231.001	6/1/00	60/208,329
United States	2750-0921P	80268.001	6/1/00	60/208,312
United States	2750-0915P	80266.001	6/2/00	60/209,338
United States	2750-0917P	80267.001	6/2/00	60/208,649
United States	2750-0926P	00233.001	6/5/00	60/208,921
United States	2750-0924P	80269.001	6/5/00	60/208,918
United States	2750-0925P	91070.001	6/5/00	60/208,917
United States	2750-0922P	91069.001	6/5/00	60/208,919
United States	2750-0927P	80270.001	6/5/00	60/208,920
United States	2750-0923P	00232.001	6/5/00	60/208,910
United States	2750-0930P	00234.001	6/8/00	60/210,012
United States	2750-0929P	91071.001	6/8/00	60/210,008
United States	2750-0931P	80271.001	6/8/00	60/210,006
United States	2750-0933P	80272.001	6/9/00	60/210,564
United States	2750-0932P	00235.001	6/9/00	60/210,670
Mexico	2750-1037F(MX)	91000.103(MX)	6/9/00	00/005,741
Mexico	2750-0928F(MX)	00033.102	6/9/00	00/005,740
Canada	2750-0928F(CA)	00033.100	6/9/00	
United States	2750-0928P	00033.003	6/9/00	09/592,459
Europe	2750-0928F(EP)	00033.101	6/12/00	00/304,943
United States	2750-0937P	91072.001	6/13/00	60/211,210
United States	2750-0935P	00237.001	6/13/00	60/211,213
United States	2750-0936P	80273.001	6/13/00	60/211,214
Canada	2750-0934F(CA)	00034.100	6/14/00	
Mexico	2750-0934F(MX)	00034.102	6/14/00	00/005,842
United States	2750-0934P	00034.002	6/14/00	09/593,710
Europe	2750-0934F(EP)	00034.101	6/14/00	00/305,026
United States	2750-0938P	00238.001	6/15/00	60/211,539
United States	2750-0940P	91074.001	6/15/00	60/211,538
United States	2750-0939P	80274.001	6/15/00	60/211,540
Europe	2750-0943F(EP)	00039.101	6/16/00	
Mexico	2750-0941F(MX)	00037.102	6/16/00	00/005,950
Europe	2750-0941F(EP)	00037.101	6/16/00	00/305,144
Canada	2750-0941F(CA)	00037.100	6/16/00	
Mexico	2750-0943F(MX)	00039.102	6/16/00	
United States	2750-0950P	80132.019	6/16/00	09/594,598
Mexico	2750-0942F(MX)	00038.102	6/16/00	
Europe	2750-0942F(EP)	00038.101	6/16/00	
United States	2750-0945P	80132.014	6/16/00	09/595,333
United States	2750-0944P	80132.013	6/16/00	09/595,330
United States	2750-0943P	00039.002	6/16/00	09/596,577
United States	2750-0947P	80132.016	6/16/00	09/595,335
United States	2750-0954P	80132.023	6/16/00	09/594,599
United States	2750-0955P	80132.024	6/16/00	09/595,331
United States	2750-0952P	80132.021	6/16/00	09/594,597

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United States	2750-0948P	80132.017	6/16/00	09/595,329
United States	2750-0942P	00038.002	6/16/00	09/595,326
Canada	2750-0942F(CA)	00038.100	6/16/00	
United States	2750-0946P	80132.015	6/16/00	09/595,328
United States	2750-0941P	00037.002	6/16/00	09/595,334
United States	2750-0949P	80132.018	6/16/00	09/595,332
Canada	2750-0943F(CA)	00039.100	6/16/00	
United States	2750-0953P	80132.022	6/16/00	09/595,298
United States	2750-0951P	80132.020	6/16/00	09/594,595
United States	2750-0958P	91075.001	6/19/00	60/212,623
United States	2750-0956P	00239.001	6/19/00	60/212,414
United States	2750-0957P	80275.001	6/19/00	60/212,649
United States	2750-0960P	80276.001	6/20/00	60/212,713
United States	2750-0961P	91076.001	6/20/00	60/212,727
United States	2750-0959P	00240.001	6/20/00	60/212,677
Europe	2750-0971F(EP)	00042.101	6/21/00	00/305,249
Mexico	2750-0971F(MX)	00042.102	6/21/00	00/006,142
Canada	2750-0971F(CA)	00042.100	6/21/00	02/309,874
United States	2750-0971P	00042.003	6/21/00	09/602,660
United States	2750-0962P	00242.001	6/22/00	60/213,271
United States	2750-0963P	80277.001	6/22/00	60/213,249
Europe	2750-0972F(EP)	00043.101	6/22/00	00/305,270
Mexico	2750-0972F(MX)	00043.102	6/22/00	00/006,625
United States	2750-0967P	91079.001	6/22/00	60/213,270
United States	2750-0964P	91077.001	6/22/00	60/213,195
United States	2750-0965P	00246.001	6/22/00	60/213,221
Canada	2750-0972F(CA)	00043.100	6/22/00	02/309,793
United States	2750-0972P	00043.002	6/22/00	09/602,152
United States	2750-0966P	80278.001	6/22/00	60/213,220
United States	2750-0973P	00044.002	6/23/00	09/602,205
Canada	2750-0973F(CA)	00044.100	6/23/00	02/309,889
Canada	2750-0974F(CA)	00042.100	6/23/00	
Europe	2750-0974F(EP)	00042.101	6/23/00	
Mexico	2750-0974F(MX)	00042.102	6/23/00	
Europe	2750-0973F(EP)	00044.101	6/23/00	00/305,305
Mexico	2750-0975F(MX)	00045.102	6/23/00	
Mexico	2750-0973F(MX)	00044.102	6/23/00	00/006,267
United States	2750-0975P	00045.002	6/23/00	09/602,016
Canada	2750-0975F(CA)	00045.100	6/23/00	
Europe	2750-0975F(EP)	00045.101	6/23/00	
United States	2750-0968P	00247.001	6/27/00	
United States	2750-1036P	80280.001	6/27/00	60/214,535
United States	2750-1035P	00248.001	6/27/00	60/214,534
United States	2750-0969P	80279.001	6/27/00	60/214,762
United States	2750-0970P	91080.001	6/27/00	60/214,524
United States	2750-0976P	00046.002	6/28/00	09/605,843
United States	2750-1039P	80281.001	6/28/00	60/214,799
Mexico	2750-0976F(MX)	00046.102	6/28/00	
Europe	2750-0976F(EP)	00046.101	6/28/00	
Canada	2750-0976F(CA)	00046.100	6/28/00	

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United States	2750-1038P	00249.001	6/28/00	60/214,800
Canada	2750-0977F(CA)	00048.100	6/29/00	
United States	2750-0977P	00048.002	6/29/00	09/606,181
Europe	2750-0977F(EP)	00048.101	6/29/00	
Mexico	2750-0977F(MX)	00048.102	6/29/00	
Mexico	2750-0981F(MX)	00052.102	6/30/00	
Canada	2750-0980F(CA)	00051.100	6/30/00	
United States	2750-0979P	00050.002	6/30/00	09/607,081
Mexico	2750-0978F(MX)	00049.102	6/30/00	
Europe	2750-0978F(EP)	00049.101	6/30/00	
Canada	2750-0978F(CA)	00049.100	6/30/00	
United States	2750-1041P	80282.001	6/30/00	60/215,127
Mexico	2750-0979F(MX)	00050.102	6/30/00	
Europe	2750-0979F(EP)	00050.101	6/30/00	
United States	2750-0978P	00049.002	6/30/00	09/608,960
Europe	2750-0980F(EP)	00051.101	6/30/00	
Mexico	2750-0980F(MX)	00051.102	6/30/00	
Europe	2750-0981F(EP)	00052.101	6/30/00	
Canada	2750-0981F(CA)	00052.100	6/30/00	
United States	2750-0981P	00052.002	6/30/00	09/609,198
United States	2750-0980P	00051.002	6/30/00	09/610,157
United States	2750-1040P	00250.001	6/30/00	60/215,775
Canada	2750-0979F(CA)	00050.100	6/30/00	
United States	2750-1043P	80283.001	7/5/00	60/216,362
United States	2750-1042P	00252.001	7/5/00	
Canada	2750-0982F(CA)	00053.100	7/6/00	
Mexico	2750-0982F(MX)	00053.102	7/6/00	
United States	2750-0982P	00053.002	7/6/00	09/611,409
Europe	2750-0982F(EP)	00053.101	7/6/00	
United States	2750-0984P	00058.002	7/7/00	09/613,547
Canada	2750-0984F(CA)	00058.100	7/7/00	
Mexico	2750-0984F(MX)	00058.102	7/7/00	
Europe	2750-0984F(EP)	00058.101	7/7/00	
Mexico	2750-0983F(MX)	00054.102	7/7/00	
Europe	2750-0983F(EP)	00054.101	7/7/00	
Canada	2750-0983F(CA)	00054.100	7/7/00	
United States	2750-0983P	00054.002	7/7/00	09/612,645
United States	2750-1044P	91081.001	7/11/00	60/217,385
United States	2750-1046P	80284.001	7/11/00	60/217,384
United States	2750-1045P	00253.001	7/11/00	60/217,476
United States	2750-1052P	80287.002	7/12/00	
Europe	2750-0985F(EP)	00059.101	7/12/00	
Canada	2750-0985F(CA)	00059.100	7/12/00	
United States	2750-0985P	00059.002	7/12/00	09/615,007
United States	2750-1050P	80286.002	7/12/00	
Mexico	2750-0985F(MX)	00059.102	7/12/00	
Canada	2750-0986F(CA)	00060.100	7/13/00	
United States	2750-1054P	80288.002	7/13/00	
Mexico	2750-0986F(MX)	00060.102	7/13/00	
Europe	2750-0986F(EP)	00060.101	7/13/00	

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United States	2750-0986P	00060.002	7/13/00	09/615,748
United States	2750-1061P	80134.018	7/14/00	09/614,450
United States	2750-1060P	80134.017	7/14/00	09/614,388
United States	2750-0987P	00061.002	7/14/00	09/617,525
Mexico	2750-0988F(MX)	00062.102	7/14/00	
Canada	2750-0987F(CA)	00061.100	7/14/00	
Europe	2750-0987F(EP)	00061.101	7/14/00	
Mexico	2750-0987F(MX)	00061.102	7/14/00	
United States	2750-0988P	00062.002	7/14/00	09/617,203
Europe	2750-0988F(EP)	00062.101	7/14/00	
United States	2750-1048P	80285.002	7/14/00	60/218,566
Canada	2750-0988F(CA)	00062.100	7/14/00	
United States	2750-1057P	80291.001	7/18/00	
United States	2750-1056P	00254.001	7/18/00	
United States	2750-1055P	91082.001	7/18/00	60/219,021
United States	2750-0989P	00064.002	7/19/00	09/620,421
Canada	2750-0989F(CA)	00064.100	7/19/00	
Europe	2750-0989F(EP)	00064.101	7/19/00	
Mexico	2750-0989F(MX)	00064.102	7/19/00	
United States	2750-1062P	80134.020	7/19/00	09/617,683
United States	2750-1063P	80134.022	7/19/00	09/617,682
United States	2750-1064P	80134.024	7/19/00	09/617,681
United States	2750-0990P	00065.002	7/20/00	09/621,323
Canada	2750-0990F(CA)	00065.100	7/20/00	
Europe	2750-0990F(EP)	00065.101	7/20/00	
United States	2750-1065P	80134.026	7/20/00	09/620,998
Mexico	2750-0990F(MX)	00065.102	7/20/00	
United States	2750-1066P	80135.004	7/20/00	09/620,978
United States	2750-1069P	80134.023	7/21/00	09/620,313
United States	2750-1071P	80134.021	7/21/00	09/620,390
United States	2750-1073P	80134.025	7/21/00	09/620,314
United States	2750-1072P	80135.003	7/21/00	09/621,900
United States	2750-0992P	00067.002	7/21/00	09/621,660
Canada	2750-0992F(CA)	00067.100	7/21/00	
Europe	2750-0992F(EP)	00067.101	7/21/00	
Europe	2750-0991F(EP)	00066.101	7/21/00	
United States	2750-1067P	80134.015	7/21/00	09/620,394
Mexico	2750-0992F(MX)	00067.102	7/21/00	
United States	2750-0993P	00069.002	7/21/00	09/621,902
Canada	2750-0993F(CA)	00069.100	7/21/00	
Europe	2750-0993F(EP)	00069.101	7/21/00	
Canada	2750-0991F(CA)	00066.100	7/21/00	
Mexico	2750-0993F(MX)	00069.102	7/21/00	
United States	2750-0991P	00066.002	7/21/00	09/621,630
United States	2750-1068P	80134.016	7/21/00	09/620,393
United States	2750-1070P	80134.019	7/21/00	09/620,111
Mexico	2750-0991F(MX)	00066.102	7/21/00	
United States	2750-1081P	80293.001	7/25/00	
United States	2750-1058P	91083.001	7/25/00	
United States	2750-1059P	00255.001	7/25/00	

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United States	2750-1080P	00256.001	7/25/00	
United States	2750-1079P	80292.001	7/25/00	
Canada	2750-0994F(CA)	00070.100	7/26/00	
Europe	2750-0994F(EP)	00070.101	7/26/00	
Mexico	2750-0994F(MX)	00070.102	7/26/00	
United States	2750-0994P	00070.002	7/26/00	09/616,628
Europe	2750-0995F(EP)	00071.101	7/27/00	
United States	2750-1075P	80136.005	7/27/00	09/628,987
United States	2750-1074P	80136.004	7/27/00	09/628,984
Mexico	2750-0995F(MX)	00071.102	7/27/00	
Canada	2750-0995F(CA)	00071.100	7/27/00	
United States	2750-0995P	00071.002	7/27/00	09/628,986
Canada	2750-0996F(CA)	00072.100	7/28/00	
Europe	2750-0996F(EP)	00072.101	7/28/00	
Mexico	2750-0996F(MX)	00072.102	7/28/00	
United States	2750-0996P	00072.002	7/28/00	09/628,552
United States	2750-1049P	80286.001	8/1/00	
United States	2750-1256P	80299.002	8/1/00	
United States	2750-1077P	80137.004	8/2/00	09/630,442
United States	2750-1076P	80137.003	8/2/00	09/628,985
Mexico	2750-0997F(MX)	00073.102	8/2/00	
Europe	2750-0997F(EP)	00073.101	8/2/00	
Canada	2750-0997F(CA)	00073.100	8/2/00	
United States	2750-0997P	00073.002	8/2/00	09/632,340
United States	2750-1051P	80287.001	8/3/00	
United States	2750-0998P	00074.002	8/3/00	09/632,349
United States	2750-1255P	80299.001	8/3/00	
United States	2750-1053P	80288.002	8/3/00	60/223,100
United States	2750-1257P	80299.003	8/3/00	
United States	2750-1258P	80299.004	8/3/00	
Mexico	2750-0998F(MX)	00074.102	8/3/00	
Europe	2750-0998F(EP)	00074.101	8/3/00	
Canada	2750-0998F(CA)	00074.100	8/3/00	
Canada	2750-1001F(CA)	00079.100	8/4/00	
Canada	2750-1000F(CA)	00077.100	8/4/00	
United States	2750-1000P	00077.002	8/4/00	09/633,191
Mexico	2750-1001F(MX)	00079.102	8/4/00	
Mexico	2750-1000F(MX)	00077.102	8/4/00	
Europe	2750-1000F(EP)	00077.101	8/4/00	
Europe	2750-1001F(EP)	00079.101	8/4/00	
United States	2750-1001P	00079.002	8/4/00	09/633,239
Mexico	2750-0999F(MX)	00076.102	8/4/00	
United States	2750-1078P	80138.003	8/4/00	09/635,640
United States	2750-0999P	00076.002	8/4/00	09/633,051
Canada	2750-0999F(CA)	00076.100	8/4/00	
Europe	2750-0999F(EP)	00076.101	8/4/00	
United States	2750-1092P	80138.004	8/4/00	09/635,643
United States	2750-1047P	80285.001	8/7/00	
United States	2750-1094P	80139.004	8/9/00	09/635,642
Mexico	2750-1002F(MX)	00080.102	8/9/00	

Country	Attorney No.	Client No.	Filed	Application No.
Europe	2750-1002F(EP)	00080.101	8/9/00	
United States	2750-1115P	92002.004	8/9/00	
United States	2750-1114P	92001.004	8/9/00	60/224,390
United States	2750-1002P	00080.002	8/9/00	09/635,277
Canada	2750-1002F(CA)	00080.100	8/9/00	
United States	2750-1093P	80139.003	8/10/00	09/635,641
Europe	2750-1005F(EP)	00083.101	8/11/00	
United States	2750-1005P	00083.002	8/11/00	09/637,563
Mexico	2750-1004F(MX)	00082.102	8/11/00	
Europe	2750-1004F(EP)	00082.101	8/11/00	
Canada	2750-1005F(CA)	00083.100	8/11/00	
Canada	2750-1004F(CA)	00082.100	8/11/00	
Mexico	2750-1005F(MX)	00083.102	8/11/00	
United States	2750-1096P	80142.004	8/11/00	09/637,780
Mexico	2750-1003F(MX)	00081.102	8/11/00	
United States	2750-1101P	80141.005	8/11/00	
United States	2750-1103P	80141.007	8/11/00	
United States	2750-1003P	00081.002	8/11/00	09/637,837
United States	2750-1004P	00082.002	8/11/00	09/636,555
Europe	2750-1003F(EP)	00081.101	8/11/00	
United States	2750-1104P	80141.008	8/11/00	
United States	2750-1102P	80141.006	8/11/00	09/637,565
Canada	2750-1003F(CA)	00081.100	8/11/00	
United States	2750-1082P	80298.001	8/14/00	
United States	2750-1083P	91084.001	8/14/00	
United States	2750-1085P	91085.001	8/15/00	
United States	2750-1084P	80300.001	8/15/00	
United States	2750-1095P	80142.003	8/16/00	09/643,672
United States	2750-1110P	80296.002	8/16/00	60/225,850
United States	2750-1108P	80295.002	8/16/00	
United States	2750-1112P	80297.002	8/16/00	
United States	2750-1106P	80294.002	8/16/00	
Europe	2750-1006F(EP)	00084.101	8/17/00	
United States	2750-1006P	00084.002	8/17/00	09/641,198
Mexico	2750-1006F(MX)	00084.102	8/17/00	
Canada	2750-1006F(CA)	00084.100	8/17/00	
Canada	2750-1008F(CA)	00086.100	8/18/00	
Canada	2750-1007F(CA)	00085.100	8/18/00	
United States	2750-1007P	00085.002	8/18/00	
Mexico	2750-1007F(MX)	00085.102	8/18/00	
United States	2750-1111P	80297.001	8/18/00	60/226,324
Europe	2750-1008F(EP)	00086.101	8/18/00	
United States	2750-1008P	00086.002	8/18/00	
Europe	2750-1007F(EP)	00085.101	8/18/00	
United States	2750-1109P	80296.001	8/18/00	60/226,323
United States	2750-1107P	80295.001	8/18/00	
mexico	2750-1009F(MX)	00087.102	8/18/00	
United States	2750-1098P	80143.004	8/18/00	09/643,671
United States	2750-1009P	00087.002	8/18/00	09/640,695
United States	2750-1105P	80294.001	8/18/00	60/226,325

Country	Attorney No.	Client No.	Filed	Application No.
Europe	2750-1009F(EP)	00087.101	8/18/00	
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United States	2750-1087P	91086.001	8/21/00	
United States	2750-1086P	80301.001	8/21/00	
United States	2750-1124P	80289.009	8/23/00	
United States	2750-1117P	80289.002	8/23/00	
United States	2750-1118P	80289.003	8/23/00	
United States	2750-1122P	80289.007	8/23/00	
United States	2750-1131P	80289.016	8/23/00	
United States	2750-1123P	80289.008	8/23/00	
United States	2750-1132P	80289.017	8/23/00	
United States	2750-1162P	80307.001	8/23/00	
Mexico	2750-1010F(MX)	00088.102	8/23/00	
Europe	2750-1010F(EP)	00088.101	8/23/00	
United States	2750-1010P	00088.002	8/23/00	09/643,854
United States	2750-1116P	80289.001	8/23/00	
United States	2750-1097P	80143.003	8/23/00	09/649,866
United States	2750-1163P	91087.001	8/23/00	
Canada	2750-1010F(CA)	00088.100	8/23/00	
United States	2750-1145P	80289.030	8/25/00	60/227,728
United States	2750-1127P	80289.012	8/25/00	
United States	2750-1144P	80289.029	8/25/00	
United States	2750-1099P	80144.003	8/25/00	09/649,868
United States	2750-1100P	80144.004	8/25/00	09/649,867
United States	2750-1140P	80289.025	8/25/00	
Mexico	2750-1013F(MX)	00091.102	8/25/00	
United States	2750-1126P	80289.011	8/25/00	
United States	2750-1151P	80289.036	8/25/00	
United States	2750-1143P	80289.028	8/25/00	
United States	2750-1137P	80289.022	8/25/00	
United States	2750-1150P	80289.035	8/25/00	
United States	2750-1121P	80289.006	8/25/00	
United States	2750-1136P	80289.021	8/25/00	
United States	2750-1135P	80289.020	8/25/00	
United States	2750-1134P	80289.019	8/25/00	
United States	2750-1120P	80289.005	8/25/00	
Mexico	2750-1012F(MX)	00090.102	8/25/00	
United States	2750-1133P	80289.018	8/25/00	
Mexico	2750-1011F(MX)	00089.102	8/25/00	
Europe	2750-1012F(EP)	00090.101	8/25/00	
Canada	2750-1012F(CA)	00090.100	8/25/00	
United States	2750-1012P	00090.002	8/25/00	
United States	2750-1147P	80289.032	8/25/00	
United States	2750-1146P	80289.031	8/25/00	
United States	2750-1149P	80289.034	8/25/00	
Canada	2750-1013F(CA)	00091.100	8/25/00	
Europe	2750-1013F(EP)	00091.101	8/25/00	
United States	2750-1148P	80289.033	8/25/00	
Europe	2750-1011F(EP)	00089.101	8/25/00	

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Country	Attorney No.	Client No.	Filed	Application No.
United States	2750-1119P	80289.004	8/25/00	60/228,029
United States	2750-1013P	00091.002	8/25/00	
United States	2750-1011P	00089.002	8/25/00	
United States	2750-1139P	80289.024	8/25/00	60/228,167
United States	2750-1128P	80289.013	8/25/00	60/227,726
United States	2750-1138P	80289.023	8/25/00	60/227,729
United States	2750-1142P	80289.027	8/25/00	60/227,733
United States	2750-1141P	80289.026	8/25/00	
United States	2750-1152P	80289.037	8/25/00	
Canada	2750-1011F(CA)	00089.100	8/25/00	
United States	2750-1226P	91088.001	8/30/00	
Europe	2750-1014F(EP)	00092.101	8/30/00	
Mexico	2750-1014F(MX)	00092.102	8/30/00	
United States	2750-1225P	80308.001	8/30/00	
United States	2750-1014P	00092.002	8/30/00	09/651,370
Canada	2750-1014F(CA)	00092.100	8/30/00	
Canada	2750-1015F(CA)	00093.100	8/31/00	
Mexico	2750-1015F(MX)	00093.102	8/31/00	
United States	2750-1088P	80302.001	8/31/00	
Europe	2750-1015F(EP)	00093.101	8/31/00	
United States	2750-1089P	80302.002	8/31/00	
United States	2750-1090P	80303.001	8/31/00	
United States	2750-1113P	80304.001	8/31/00	
United States	2750-1091P	80303.002	8/31/00	
United States	2750-1015P	00093.002	8/31/00	09/653,466
Canada	2750-1016F(CA)	00094.100	9/1/00	
Mexico	2750-1016F(MX)	00094.102	9/1/00	
United States	2750-1016P	00094.002	9/1/00	09/654,547
Europe	2750-1016F(EP)	00094.101	9/1/00	
United States	2750-1159P	80305.002	9/6/00	
United States	2750-1228P	91089.001	9/6/00	
United States	2750-1160P	80306.001	9/6/00	
United States	2750-1158P	80305.001	9/6/00	
United States	2750-1157P	80304.002	9/6/00	
United States	2750-1161P	80306.002	9/6/00	
United States	2750-1227P	80309.001	9/6/00	
United States	2750-1017P	00095.002	9/7/00	
Europe	2750-1017F(EP)	00095.101	9/7/00	
Mexico	2750-1017F(MX)	00095.102	9/7/00	
Canada	2750-1017F(CA)	00095.100	9/7/00	
Mexico	2750-1018F(MX)	00096.102	9/8/00	
United States	2750-1018P	00096.002	9/8/00	09/657,569
Canada	2750-1018F(CA)	00096.100	9/8/00	
Europe	2750-1018F(EP)	00096.101	9/8/00	
United States	2750-1230P	91090.001	9/13/00	
Mexico	2750-1019F(MX)	00098.102	9/13/00	
Europe	2750-1019F(EP)	00098.101	9/13/00	
Canada	2750-1019F(CA)	00098.100	9/13/00	
United States	2750-1019P	00098.002	9/13/00	09/660,883
United States	2750-1229P	80310.001	9/13/00	

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United States	2750-1021P	00101.002	9/15/00	
Canada	2750-1021F(CA)	00101.100	9/15/00	
Europe	2750-1021F(EP)	00101.101	9/15/00	
United States	2750-1231P	80311.001	9/15/00	
Mexico	2750-1021F(MX)	00101.102	9/15/00	
United States	2750-1020P	00099.002	9/15/00	
Canada	2750-1020F(CA)	00099.100	9/15/00	
Europe	2750-1020F(EP)	00099.101	9/15/00	
Mexico	2750-1020F(MX)	00099.102	9/15/00	
United States	2750-1232P	91091.001	9/15/00	
United States	2750-1233P	80312.001	9/18/00	
United States	2750-1234P	91092.001	9/18/00	
United States	2750-1253P	80313.001	9/20/00	
United States	2750-1254P	91093.001	9/20/00	
United States	2750-1022P	00102.002	9/20/00	
Canada	2750-1022F(CA)	00102.100	9/20/00	
Europe	2750-1022F(EP)	00102.101	9/20/00	
Mexico	2750-1022F(MX)	00102.102	9/20/00	
United States	2750-1261P	91095.001	9/21/00	
United States	2750-1260P	91094.001	9/21/00	
United States	2750-1259P	80314.001	9/21/00	
United States	2750-1023P	00103.002	9/22/00	
Canada	2750-1025F(CA)	00105.100	9/22/00	
Europe	2750-1023F(EP)	00103.101	9/22/00	
United States	2750-1025P	00105.002	9/22/00	
Canada	2750-1023F(CA)	00103.100	9/22/00	
Mexico	2750-1024F(MX)	00104.102	9/22/00	
Europe	2750-1024F(EP)	00104.101	9/22/00	
Canada	2750-1024F(CA)	00104.100	9/22/00	
United States	2750-1024P	00104.002	9/22/00	
Mexico	2750-1025F(MX)	00105.102	9/22/00	
Europe	2750-1025F(EP)	00105.101	9/22/00	
Mexico	2750-1023F(MX)	00103.102	9/22/00	
United States	2750-1264P	80316.001	9/25/00	
United States	2750-1265P	91097.001	9/25/00	
United States	2750-1263P	91096.001	9/25/00	
United States	2750-1262P	80315.001	9/25/00	
United States	2750-1266P	80317.001	9/26/00	
United States	2750-1267P	91098.001	9/27/00	
United States	2750-1026P	00106.002	9/28/00	
United States	2750-1268P	91078.001	9/28/00	
United States	2750-1269P	91099.001	9/29/00	
United States	2750-1027P	00107.002	9/29/00	
United States	2750-1270P	80318.001	10/2/00	
United States	2750-1271P	91100.001	10/2/00	
United States	2750-1028P	00108.002	10/4/00	
United States	2750-1272P	80319.001	10/4/00	
United States	2750-1273P	91101.001	10/4/00	
United States	2750-1274P	80320.001	10/5/00	
United States	2750-1029P	00109.002	10/5/00	

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United States	2750-1030P	00110.002	10/6/00	
United States	2750-1031P	00111.002	10/6/00	
United States	2750-1032P	00112.002	10/6/00	
United States	2750-1277P	80322.001	10/10/00	
United States	2750-1276P	91102.001	10/10/00	
United States	2750-1275P	80321.001	10/10/00	
United States	2750-1278P	91103.001	10/10/00	
United States	2750-1280P	91104.001	10/11/00	
United States	2750-1279P	80323.001	10/11/00	
United States	2750-1033P	00113.002	10/12/00	
United States	2750-1281P	80324.001	10/17/00	
United States	2750-1282P	91105.001	10/17/00	
United States	2750-1034P	00116.002	10/18/00	

All applications listed in the table above are expressly incorporated herein by reference in their entirety and for all purposes.

The SDFs of the invention can also be used as probes to search for genes that are related to the SDF within a species. Such related genes are typically considered to be members of a gene family. In such a case, the sequence similarity will often be concentrated into one or a few fragments of the sequence. The fragments of similar sequence that define the gene family typically encode a fragment of a protein or RNA that has an enzymatic or structural function. The percentage of identity in the amino acid sequence of the domain that defines the gene family is preferably at least 70%, more preferably 80 to 95%, most preferably 85 to 99%. To search for members of a gene family within a species, a low stringency hybridization is usually performed, but this will depend upon the size, distribution and degree of sequence divergence of domains that define the gene family. SDFs encompassing regulatory regions can be used to identify coordinately expressed genes by using the regulatory region sequence of the SDF as a probe.

In the instances where the SDFs are identified as being expressed from genes that confer a particular phenotype, then the SDFs can also be used as probes to assay plants of different species for those phenotypes.

I.C.   Methods to Inhibit Gene Expression

The nucleic acid molecules of the present invention can be used to inhibit gene transcription and/or translation. Example of such methods include, without limitation:

Antisense Constructs;

Ribozyme Constructs;

Chimeraplast Constructs;

Co-Suppression;

Transcriptional Silencing; and

Other Methods of Gene Expression.

5

C.1   Antisense

In some instances it is desirable to suppress expression of an endogenous or exogenous gene. A well-known instance is the FLAVOR-SAVOR™ tomato, in which the

10   gene encoding ACC synthase is inactivated by an antisense approach, thus delaying softening of the fruit after ripening. See for example, U.S. Patent No. 5,859,330; U.S. Patent No. 5,723,766; Oeller, et al, *Science,* 254:437-439(1991); and Hamilton et al, *Nature,* 346:284-287 (1990). Also, timing of flowering can be controlled by suppression of the *FLOWERING LOCUS C (FLC)*; high levels of this transcript are associated with late flowering, while

15   absence of *FLC* is associated with early flowering (S.D. Michaels et al., *Plant Cell* 11:949 (1999). Also, the transition of apical meristem from production of leaves with associated shoots to flowering is regulated by *TERMINAL FLOWER1, APETALA1* and *LEAFY*. Thus, when it is desired to induce a transition from shoot production to flowering, it is desirable to suppress *TFL1* expression (S.J. Liljegren, *Plant Cell* 11:1007 (1999)). As another instance,

20   arrested ovule development and female sterility result from suppression of the ethylene forming enzyme but can be reversed by application of ethylene (D. De Martinis et al., *Plant Cell* 11:1061 (1999)). The ability to manipulate female fertility of plants is useful in increasing fruit production and creating hybrids.

In the case of polynucleotides used to inhibit expression of an endogenous gene, the

25   introduced sequence need not be perfectly identical to a sequence of the target endogenous gene. The introduced polynucleotide sequence will typically be at least substantially identical to the target endogenous sequence.

Some polynucleotide SDFs in Table 1 represent sequences that are expressed in corn,wheat, rice, soybean *Arabidopsis* and/or other plants. Thus the invention includes using

30   these sequences to generate antisense constructs to inhibit translation and/or degradation of transcripts of said SDFs, typically in a plant cell.

To accomplish this, a polynucleotide segment from the desired gene that can hybridize to the mRNA expressed from the desired gene (the   antisense segment") is operably linked to a promoter such that the antisense strand of RNA will be transcribed when the construct is present

in a host cell. A regulated promoter can be used in the construct to control transcription of the antisense segment so that transcription occurs only under desired circumstances.

The antisense segment to be introduced generally will be substantially identical to at least a fragment of the endogenous gene or genes to be repressed. The sequence, however, need

5   not be perfectly identical to inhibit expression. Further, the antisense product may hybridize to the untranslated region instead of or in addition to the coding sequence of the gene. The vectors of the present invention can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene.

For antisense suppression, the introduced antisense segment sequence also need not

10   be full length relative to either the primary transcription product or the fully processed mRNA. Generally, a higher percentage of sequence identity can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 30 or 40 nucleotides and the full length of the

15   transcript canbe used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of at least about 500 nucleotides is especially preferred.

C.2.   Ribozymes

20   It is also contemplated that gene constructs representing ribozymes and based on the SDFs in TABLE 1 are an object of the invention. Ribozymes can also be used to inhibit expression of genes by suppressing the translation of the mRNA into a polypeptide. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA.

25   In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

A number of classes of ribozymes have been identified. One class of ribozymes is

30   derived from a number of small circular RNAs, which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus,

solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. *Nature,* <u>334</u>:585 (1988).

Like the antisense constructs above, the ribozyme sequence fragment necessary for pairing need not be identical to the target nucleotides to be cleaved, nor identical <u>to</u> the

5   sequences in TABLE 1. Ribozymes may be constructed by combining the ribozyme sequence and some fragment of the target gene which would allow recognition of the target gene mRNA by the resulting ribozyme molecule. Generally, the sequence in the ribozyme capable of binding to the target sequence exhibits a percentage of sequence identity with at least 80%, preferably with at least 85%, more preferably with at least 90% and most preferably

10   with at least 95%, even more preferably, with at least 96%, 97%, 98% or 99% sequence identity to some fragment of a sequence in TABLE 1 or the complement thereof. The ribozyme can be equally effective in inhibiting mRNA translation by cleaving either in the untranslated or coding regions. Generally, a higher percentage of sequence identity can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not

15   have the same intron or exon pattern, and homology of non-coding segments may be equally effective.

C.3. Chimeraplasts

The SDFs of the invention, such as those described by Table 1, can also be used to construct chimeraplasts that can be introduced into a cell to produce at least one specific

20   nucleotide change in a sequence corresponding to the SDF of the invention. A chimeraplast is an oligonucleotide comprising DNA and/or RNA that specifically hybridizes to a target region in a manner which creates a mismatched base-pair. This mismatched base-pair signals the cell's repair enzyme machinery which acts on the mismatched region resulting in the replacement, insertion or deletion of designated nucleotide(s). The altered sequence is then

25   expressed by the cell's normal cellular mechanisms. Chimeraplasts can be designed to repair mutant genes, modify genes, introduce site-specific mutations, and/or act to interrupt or alter normal gene function (US Pat. Nos. 6,010,907 and 6,004,804; and PCT Pub. No. WO99/58723 and WO99/07865).

C.4.   <u>Sense Suppression</u>

The SDFs of Table 1 of the present invention are also useful to modulate gene

30   expression by sense suppression. Sense suppression represents another method of gene

suppression by introducing at least one exogenous copy or fragment of the endogenous sequence to be suppressed.

Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter into the chromosome of a plant or by a self-replicating

5   virus has been shown to be an effective means by which to induce degradation of mRNAs of target genes. For an example of the use of this method to modulate expression of endogenous genes *see*, Napoli et al., *The Plant Cell* 2:279 (1990), and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184. Inhibition of expression may require some transcription of the introduced sequence.

10   For sense suppression, the introduced sequence generally will be substantially identical to the endogenous sequence intended to be inactivated. The minimal percentage of sequence identity will typically be greater than about 65%, but a higher percentage of sequence identity might exert a more effective reduction in the level of normal gene products. Sequence identity of more than about 80% is preferred, though about 95% to absolute identity would be most

15   preferred. As with antisense regulation, the effect would likely apply to any other proteins within a similar family of genes exhibiting homology or substantial homology to the suppressing sequence.

C.5. Transcriptional Silencing

20   The nucleic acid sequences of the invention, including the SDFs of Table 1, and fragments thereof, contain sequences that can be inserted into the genome of an organism resulting in transcriptional silencing. Such regulatory sequences need not be operatively linked to coding sequences to modulate transcription of a gene. Specifically, a promoter sequence without any other element of a gene can be introduced into a genome to transcriptionally silence

25   an endogenous gene (see, for example, Vaucheret, H et al. (1998) The Plant Journal 16: 651-659). As another example, triple helices can be formed using oligonucleotides based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The oligonucleotide can be delivered to the host cell and can bind to the promoter in the genome to form a triple helix and prevent transcription. An oligonucleotide of interest is one that can bind

30   to the promoter and block binding of a transcription factor to the promoter. In such a case, the oligonucleotide can be complementary to the sequences of the promoter that interact with transcription binding factors.

C.6. Other Methods to Inhibit Gene Expression

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Low frequency homologous recombination can be used to target a polynucleotide insert to a gene by flanking the polynucleotide insert with sequences that are substantially similar to the gene to be disrupted. Sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto can be used for homologous recombination.

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* 13:152 (1997). In this method, screening for clones from a library containing random insertions is preferred to identifying those that have polynucleotides inserted into the gene of interest. Such screening can be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or R_1 plants having a desired phenotype.

I.D. Methods of Functional Analysis

The constructs described in the methods under I.C. above can be used to determine the function of the polypeptide encoded by the gene that is targeted by the constructs.

Down-regulating the transcription and translation of the targeted gene in the host cell or organisms, such as a plant, may produce phenotypic changes as compared to a wild-type cell or organism. In addition, *in vitro* assays can be used to determine if any biological activity, such as calcium flux, DNA transcription, nucleotide incorporation, etc., are being modulated by the down-regulation of the targeted gene.

Coordinated regulation of sets of genes, e.g., those contributing to a desired polygenic trait, is sometimes necessary to obtain a desired phenotype. SDFs of the invention representing transcription activation and DNA binding domains can be assembled into hybrid transcriptional activators. These hybrid transcriptional activators can be used with their corresponding DNA elements (i.e., those bound by the DNA-binding SDFs) to effect coordinated expression of desired genes (J.J. Schwarz et al., *Mol. Cell. Biol.* 12:266 (1992), A. Martinez et al., *Mol. Gen. Genet.* 261:546 (1999)).

The SDFs of the invention can also be used in the two-hybrid genetic systems to identify networks of protein-protein interactions (L. McAlister-Henn et al., *Methods* 19:330 (1999), J.C. Hu et al., *Methods* 20:80 (2000), M. Golovkin et al., *J. Biol. Chem.* 274:36428

(1999), K. Ichimura et al., *Biochem. Biophys. Res. Comm.* <u>253</u>:532 (1998)). The SDFs of the invention can also be used in various expression display methods to identify important protein-DNA interactions (e.g. B. Luo et al., *J. Mol. Biol.* <u>266</u>:479 (1997)).

I.E.   Promoters

5   The SDFs of the invention are also useful as structural or regulatory sequences in a construct for modulating the expression of the corresponding gene in a plant or other organism, *e.g.* a symbiotic bacterium. For example, promoter sequences associated to SDFs of Table 1 of the present invention can be useful in directing expression of coding sequences either as constitutive promoters or to direct expression in particular cell types, tissues, or organs or in

10   response to environmental stimuli.

         With respect to the SDFs of the present invention a promoter is likely to be a relatively small portion of a genomic DNA (gDNA) sequence located in the first 2000 nucleotides upstream from an initial exon identified in a gDNA sequence or initial ATG" or methionine

15   codon or translational start site in a corresponding cDNA sequence. Such promoters are more likely to be found in the first 1000 nucleotides upstream of an initial ATG or methionine codon or translational start site of a cDNA sequence corresponding to a gDNA sequence. In particular, the promoter is usually located upstream of the transcription start site. The fragments of a particular gDNA sequence that function as elements of a promoter in a plant cell will preferably

20   be found to hybridize to gDNA sequences presented and described in Table 1 at medium or high stringency, relevant to the length of the probe and its base composition.

         Promoters are generally modular in nature. Promoters can consist of a basal promoter that functions as a site for assembly of a transcription complex comprising an RNA polymerase, for example RNA polymerase II. A typical transcription complex will include additional factors

25   such as $TF_{II}B$, $TF_{II}D$, and $TF_{II}E$. Of these, $TF_{II}D$ appears to be the only one to bind DNA directly.   The promoter might also contain one or more enhancers and/or suppressors that function as binding sites for additional transcription factors that have the function of modulating the level of transcription with respect to tissue specificity and of transcriptional responses to particular environmental or nutritional factors, and the like.

30   Short DNA sequences representing binding sites for proteins can be separated from each other by intervening sequences of varying length. For example, within a particular functional module, protein binding sites may be constituted by regions of 5 to 60, preferably 10 to 30, more

preferably 10 to 20 nucleotides. Within such binding sites, there are typically 2 to 6 nucleotides that specifically contact amino acids of the nucleic acid binding protein. The protein binding sites are usually separated from each other by 10 to several hundred nucleotides, typically by 15 to 150 nucleotides, often by 20 to 50 nucleotides. DNA binding sites in promoter elements often

5   display dyad symmetry in their sequence. Often elements binding several different proteins, and/or a plurality of sites that bind the same protein, will be combined in a region of 50 to 1,000 basepairs.

      Elements that have transcription regulatory function can be isolated from their corresponding endogenous gene, or the desired sequence can be synthesized, and recombined in

10   constructs to direct expression of a coding region of a gene in a desired tissue-specific, temporal-specific or other desired manner of inducibility or suppression. When hybridizations are performed to identify or isolate elements of a promoter by hybridization to the long sequences presented in TABLE 1, conditions are adjusted to account for the above-described nature of promoters. For example short probes, constituting the element sought, are preferably used under

15   low temperature and/or high salt conditions. When long probes, which might include several promoter elements are used, low to medium stringency conditions are preferred when hybridizing to promoters across species.

      If a nucleotide sequence of an SDF, or part of the SDF, functions as a promoter or fragment of a promoter, then nucleotide substitutions, insertions or deletions that do not

20   substantially affect the binding of relevant DNA binding proteins would be considered equivalent to the exemplified nucleotide sequence. It is envisioned that there are instances where it is desirable to decrease the binding of relevant DNA binding proteins to silence or down-regulate a promoter, or conversely to increase the binding of relevant DNA binding proteins to enhance or up-regulate a promoter and vice versa. In such instances,

25   polynucleotides representing changes to the nucleotide sequence of the DNA-protein contact region by insertion of additional nucleotides, changes to identity of relevant nucleotides, including use of chemically-modified bases, or deletion of one or more nucleotides are considered encompassed by the present invention. In addition, fragments of the promoter sequences described by Table 1 and variants thereof can be fused with other promoters or

30   fragments to facilitate transcription and/or transcription in specific type of cells or under specific conditions.

      Promoter function can be assayed by methods known in the art, preferably by measuring activity of a reporter gene operatively linked to the sequence being tested for

promoter function. Examples of reporter genes include those encoding luciferase, green fluorescent protein, GUS, neo, cat and bar.

I.F. UTRs and Junctions

Polynucleotides comprising untranslated (UTR) sequences and intron/exon junctions are

5 also within the scope of the invention. UTR sequences include introns and 5' or 3' untranslated regions (5' UTRs or 3' UTRs). Fragments of the sequences shown in TABLE 1 can comprise UTRs and intron/exon junctions.

These fragments of SDFs, especially UTRs, can have regulatory functions related to, for example, translation rate and mRNA stability. Thus, these fragments of SDFs can be isolated

10 for use as elements of gene constructs for regulated production of polynucleotides encoding desired polypeptides.

Introns of genomic DNA segments might also have regulatory functions. Sometimes regulatory elements, especially transcription enhancer or suppressor elements, are found within introns. Also, elements related to stability of heteronuclear RNA and efficiency of

15 splicing and of transport to the cytoplasm for translation can be found in intron elements. Thus, these segments can also find use as elements of expression vectors intended for use to transform plants.

Just as with promoters UTR sequences and intron/exon junctions can vary from those shown in TABLE 1. Such changes from those sequences preferably will not affect the

20 regulatory activity of the UTRs or intron/exon junction sequences on expression, transcription, or translation unless selected to do so. However, in some instances, down- or up-regulation of such activity may be desired to modulate traits or phenotypic or *in vitro* activity.

I.G. Coding Sequences

25 Isolated polynucleotides of the invention can include coding sequences that encode polypeptides comprising an amino acid sequence encoded by sequences in TABLE 1 or an amino acid sequence presented in TABLE 1.

A nucleotide sequence encodes a polypeptide if a cell (or a cell free *in vitro* system) expressing that nucleotide sequence produces a polypeptide having the recited amino acid

30 sequence when the nucleotide sequence is transcribed and the primary transcript is subsequently processed and translated by a host cell (or a cell free *in vitro* system) harboring the nucleic acid. Thus, an isolated nucleic acid that encodes a particular amino acid sequence

can be a genomic sequence comprising exons and introns or a cDNA sequence that represents the product of splicing thereof. An isolated nucleic acid encoding an amino acid sequence also encompasses heteronuclear RNA, which contains sequences that are spliced out during expression, and mRNA, which lacks those sequences.

Coding sequences can be constructed using chemical synthesis techniques or by isolating coding sequences or by modifying such synthesized or isolated coding sequences as described above.

In addition to coding sequences encoding the polypeptide sequences of TABLE 1, which are native to corn, *Arabidopsis*, soybean, rice, wheat, and other plants the isolated polynucleotides can be polynucleotides that encode variants, fragments, and fusions of those native proteins. Such polypeptides are described below in part II.

In variant polynucleotides generally, the number of substitutions, deletions or insertions is preferably less than 20%, more preferably less than 15%; even more preferably less than 10%, 5%, 3% or 1% of the number of nucleotides comprising a particularly exemplified sequence. It is generally expected that non-degenerate nucleotide sequence changes that result in 1 to 10, more preferably 1 to 5 and most preferably 1 to 3 amino acid insertions, deletions or substitutions will not greatly affect the function of an encoded polypeptide. The most preferred embodiments are those wherein 1 to 20, preferably 1 to 10, most preferably 1 to 5 nucleotides are added to, deleted from and/or substituted in the sequences specifically disclosed in TABLE 1.

Insertions or deletions in polynucleotides intended to be used for encoding a polypeptide preferably preserve the reading frame. This consideration is not so important in instances when the polynucleotide is intended to be used as a hybridization probe.

II. Polypeptides and Proteins

IIA. Native polypeptides and proteins

Polypeptides within the scope of the invention include both native proteins as well as variants, fragments, and fusions thereof. Polypeptides of the invention are those encoded by any of the six reading frames of sequences shown in TABLE 1, preferably encoded by the three frames reading in the 5' to 3' direction of the sequences as shown.

Native polypeptides include the proteins encoded by the sequences shown in TABLE 1. Such native polypeptides include those encoded by allelic variants.

Polypeptide and protein variants will exhibit at least 75% sequence identity to those native polypeptides of TABLE 1. More preferably, the polypeptide variants will exhibit at least 85% sequence identity; even more preferably, at least 90% sequence identity; more preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity. Fragments of polypeptide or fragments

5   of polypeptides will exhibit similar percentages of sequence identity to the relevant fragments of the native polypeptide. Fusions will exhibit a similar percentage of sequence identity in that fragment of the fusion represented by the variant of the native peptide.

Furthermore, polypeptide variants will exhibit at least one of the functional properties of the native protein. Such properties include, without limitation, protein interaction, DNA

10   interaction, biological activity, immunological activity, receptor binding, signal transduction, transcription activity, growth factor activity, secondary structure, three-dimensional structure, etc. As to properties related to *in vitro* or *in vivo* activities, the variants preferably exhibit at least 60% of the activity of the native protein; more preferably at least 70%, even more preferably at least 80%, 85%, 90% or 95% of at least one activity of the native protein.

15   One type of variant of native polypeptides comprises amino acid substitutions, deletions and/or insertions. Conservative substitutions are preferred to maintain the function or activity of the polypeptide.

Within the scope of percentage of sequence identity described above, a polypeptide of the invention may have additional individual amino acids or amino acid sequences inserted into

20   the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof. Likewise, some of the amino acids or amino acid sequences may be deleted from the polypeptide.

A.1 Antibodies

Isolated polypeptides can be utilized to produce antibodies. Polypeptides of the invention can generally be used, for example, as antigens for raising antibodies by known techniques. The resulting antibodies are useful as reagents for determining the distribution of the antigen protein within the tissues of a plant or within a cell of a plant. The antibodies are also useful for examining the production level of proteins in various tissues, for example in a wild-type plant or following genetic manipulation of a plant, by methods such as Western blotting.

Antibodies of the present invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the polypeptides of the invention are first used to

25   immunize a suitable animal, such as a mouse, rat, rabbit, or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the

availability of labeled anti-rabbit and anti-goat antibodies as detection reagents. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically

5   sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization.

Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic

10   container, incubating the blood at 25°C for one hour, followed by incubating the blood at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000xg for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the method of Kohler and Milstein, *Nature* 256: 495 (1975), or modification thereof. Typically, a mouse or rat is immunized as described

15   above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells can be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate, or well, coated with the protein antigen. B-cells producing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with

20   the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected Mab-

25   secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

Other methods for sustaining antibody-producing B-cell clones, such as by EBV transformation, are known.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using

30   conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase

is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TNB) to a blue

pigment, quantifiable with a spectrophotometer.

A.2 In Vitro Applications of Polypeptides

5   Some polypeptides of the invention will have enzymatic activities that are useful *in vitro*.

For example, the soybean trypsin inhibitor (Kunitz) family is one of the numerous families of

proteinase inhibitors. It comprises plant proteins which have inhibitory activity against serine

proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases.

Thus, these peptides find *in vitro* use in protein purification protocols and perhaps in

therapeutic settings requiring topical application of protease inhibitors.

10   Delta-aminolevulinic acid dehydratase (EC 4.2.1.24) (ALAD) catalyzes the second

step in the biosynthesis of heme, the condensation of two molecules of 5-aminolevulinate to

form porphobilinogen and is also involved in chlorophyll biosynthesis(Kaczor et al. (1994)

Plant Physiol. 1-4: 1411-7; Smith (1988) Biochem. J. 249: 423-8; Schneider (1976) Z.

naturforsch. [C] 31: 55-63). Thus, ALAD proteins can be used as catalysts in synthesis of

15   heme derivatives. Enzymes of biosynthetic pathways generally can be used as catalysts for *in*

vitro synthesis of the compounds representing products of the pathway.

Polypeptides encoded by SDFs of the invention can be engineered to provide

purification reagents to identify and purify additional polypeptides that bind to them. This

allows one to identify proteins that function as multimers or elucidate signal transduction or

20   metabolic pathways. In the case of DNA binding proteins, the polypeptide can be used in a

similar manner to identify the DNA determinants of specific binding (S. Pierrou et al., *Anal.*

Biochem. 229:99 (1995), S. Chusacultanachai et al., *J. Biol. Chem.* 274:23591 (1999), Q. Lin

et al., *J. Biol. Chem.* 272:27274 (1997)).

II.B. POLYPEPTIDE VARIANTS, FRAGMENTS, AND FUSIONS

25   Generally, variants, fragments, or fusions of the polypeptides encoded by the SDFs of

the invention can exhibit at least one of the activities of the identified domains and/or related

polypeptides described in Table 1 corresponding to the SDF of interest.

II.B .(1) Variants

A type of variant of the native polypeptides comprises amino acid substitutions.

30   Conservative substitutions, described above (see II.), are preferred to maintain the function or

activity of the polypeptide. Such substitutions include conservation of charge, polarity, hydrophobicity, size, etc. For example, one or more amino acid residues within the sequence can be substituted with another amino acid of similar polarity that acts as a functional equivalent, for example providing a hydrogen bond in an enzymatic catalysis. Substitutes for an

5   amino acid within an exemplified sequence are preferably made among the members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine.

10   The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Within the scope of percentage of sequence identity described above, a polypeptide of the invention may have additional individual amino acids or amino acid sequences inserted into the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof. Likewise, some of the amino acids or amino acid sequences may be deleted from the

15   polypeptide. Amino acid substitutions may also be made in the sequences; conservative substitutions being preferred.

One preferred class of variants are those that comprise (1) the domain of an encoded polypeptide and/or (2) residues conserved between the encoded polypeptide and related polypeptides. For this class of variants, the encoded polypeptide sequence is changed

20   by insertion, deletion, or substitution at positions flanking the domain and/or conserved residues.

Another class of variants includes those that comprise an encoded polypeptide sequence that is changed in the domain or conserved residues by a conservative substitution.

Yet another class of variants includes those that lack one of the *in vitro*

25   activities, or structural features of the encoded polypeptides. One example is polypeptides or proteins produced from genes comprising dominant negative mutations. Such a variant may comprise an encoded polypeptide sequence with non-conservative changes in a particular domain or group of conserved residues.

II.A.(2)   FRAGMENTS

30   Fragments of particular interest are those that comprise a domain identified for a polypeptide encoded by an SDF of the instant invention and variants thereof. Also, fragments that comprise at least one region of residues conserved between an SDF encoded polypeptide

and its related polypeptides are of great interest. Fragments are sometimes useful as polypeptides corresponding to genes comprising dominant negative mutations are.

II.A.(3)FUSIONS

Of interest are chimeras comprising (1) a fragment of the SDF encoded

5 polypeptide or variants thereof of interest and (2) a fragment of a polypeptide comprising the same domain. For example, an AP2 helix encoded by a SDF of the invention fused to second AP2 helix from ANT protein, which comprises two AP2 helices. The present invention also encompasses fusions of SDF encoded polypeptides, variants, or fragments thereof fused with related proteins or fragments thereof.

10 **DEFINITION OF DOMAINS**

The polypeptides of the invention may possess identifying domains. In addition, the domains within the SDF encoded polypeptide can be defined by the region that exhibits at least 70% sequence identity with the consensus sequences listed in the detailed description below of each of the domains.

15 The majority of the protein domain descriptions given in the protein domain table are obtained from Prosite, (http//www.expasy.ch/prosite/), and Pfam, (http//pfam.wustl.edu/browse.shtml).

A. Activities of Polypeptides Comprising Signal Peptides

20 Polypeptides comprising signal peptides are a family of proteins that are typically targeted to (1) a particular organelle or intracellular compartment, (2) interact with a particular molecule or (3) for secretion outside of a host cell. Example of polypeptides comprising signal peptides include, without limitation, secreted proteins, soluble proteins, receptors, proteins retained in the ER, etc.

25

These proteins comprising signal peptides are useful to modulate ligand-receptor interactions, cell-to-cell communication, signal transduction, intracellular communication, and activities and/or chemical cascades that take part in an organism outside or within of any particular cell.

30

One class of such proteins are soluble proteins which are transported out of the cell. These proteins can act as ligands that bind to receptor to trigger signal transduction or to permit communication between cells.

Another class is receptor proteins which also comprise a retention domain that lodges the receptor protein in the membrane when the cell transports the receptor to the surface of the cell. Like the soluble ligands, receptors can also modulate signal transduction and

5   communication between cells.

In addition the signal peptide itself can serve as a ligand for some receptors. An example is the interaction of the ER targeting signal peptide with the signal recognition particle (SRP). Here, the SRP binds to the signal peptide, halting translation, and the

10   resulting SRP complex then binds to docking proteins located on the surface of the ER, prompting transfer of the protein into the ER.

A description of signal peptide residue composition is described below in Subsection IV.C.1.

III. Methods of Modulating Polypeptide Production

It is contemplated that polynucleotides of the invention can be incorporated into a host cell or in-vitro system to modulate polypeptide production. For instance, the SDFs prepared as described herein can be used to prepare expression cassettes useful in a number of techniques for suppressing or enhancing expression.

5   An example are polynucleotides comprising sequences to be transcribed, such as coding sequences, of the present invention can be inserted into nucleic acid constructs to modulate polypeptide production. Typically, such sequences to be transcribed are heterologous to at least one element of the nucleic acid construct to generate a chimeric gene or construct.

10   Another example of useful polynucleotides are nucleic acid molecules comprising regulatory sequences of the present invention. Chimeric genes or constructs can be generated when the regulatory sequences of the invention linked to heterologous sequences in a vector construct. Within the scope of invention are such chimeric gene and/or constructs.

Also within the scope of the invention are nucleic acid molecules, whereof at least a part

15   or fragment of these DNA molecules are presented in TABLE 1 of the present application, and wherein the coding sequence is under the control of its own promoter and/or its own regulatory elements. Such molecules are useful for transforming the genome of a host cell or an organism regenerated from said host cell for modulating polypeptide production.

Additionally, a vector capable of producing the oligonucleotide can be inserted into the

20   host cell to deliver the oligonucleotide.

More detailed description of components to be included in vector constructs are described both above and below.

Whether the chimeric vectors or native nucleic acids are utilized, such polynucleotides can be incorporated into a host cell to modulate polypeptide production.

25   Native genes and/or nucleic acid molecules can be effective when exogenous to the host cell.

Methods of modulating polypeptide expression includes, without limitation:

Suppression methods, such as

         Antisense

         Ribozymes

30   Co-suppression

         Insertion of Sequences into the Gene to be Modulated

         Regulatory Sequence Modulation.

as well as Methods for Enhancing Production, such as

Insertion of Exogenous Sequences; and

Regulatory Sequence Modulation.

III.A. Suppression

5   Expression cassettes of the invention can be used to suppress expression of

endogenous genes which comprise the SDF sequence. Inhibiting expression can be useful,

for instance, to tailor the ripening characteristics of a fruit (Oeller et al., *Science* 254:437

(1991)) or to influence seed size (WO98/07842) or to provoke cell ablation (Mariani et al.,

Nature 357: 384-387 (1992).

10   As described above, a number of methods can be used to inhibit gene expression in

plants, such as antisense, ribozyme, introduction of exogenous genes into a host cell,

insertion of a polynucleotide sequence into the coding sequence and/or the promoter of the

endogenous gene of interest, and the like.

III.A.1. Antisense

15   An expression cassette as described above can be transformed into host cell or

plant to produce an antisense strand of RNA. For plant cells, antisense RNA inhibits gene

expression by preventing the accumulation of mRNA which encodes the enzyme of interest, *see*,

e.g., Sheehy et al., *Proc. Nat. Acad. Sci.* USA, 85:8805 (1988), and Hiatt et al., U.S. Patent No.

4,801,340.

III.A.2. Ribozymes

20   Similarly, ribozyme constructs can be transformed into a plant to cleave mRNA

and down-regulate translation.

III.A.3. Co-Suppression

Another method of suppression is by introducing an exogenous copy of the gene

25   to be suppressed. Introduction of expression cassettes in which a nucleic acid is configured in

the sense orientation with respect to the promoter has been shown to prevent the accumulation of

mRNA. A detailed description of this method is described above.

III.A.4.   Insertion of Sequences into the Gene to be Modulated

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Homologous recombination could be used to target a polynucleotide insert to a gene using the Cre-Lox system (A.C. Vergunst et al., *Nucleic Acids Res.* 26:2729 (1998), A.C.

5   Vergunst et al., *Plant Mol. Biol.* 38:393 (1998), H. Albert et al., *Plant J.* 7:649 (1995)).

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* 13:152 (1997). In this method, screening for clones from a library containing random insertions is preferred for identifying those that have polynucleotides inserted into the gene of interest. Such screening can

10   be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or any transgenic plants having a desired phenotype.

III.A.5. Regulatory SequenceModulation

The SDFs described in Table 1, and fragments thereof are examples of

15   nucleotides of the invention that contain regulatory sequences that can be used to suppress or inactivate transcription and/or translation from a gene of interest as discussed in I.C.5.

III.A.6. Genes Comprising Dominant-Negative Mutations

When suppression of production of the endogenous, native protein is desired it

20   is often helpful to express a gene comprising a dominant negative mutation. Production of protein variants produced from genes comprising dominant negative mutations is a useful tool for research Genes comprising dominant negative mutations can produce a variant polypeptide which is capable of competing with the native polypeptide, but which does not produce the native result. Consequently, over expression of genes comprising these mutations

25   can titrate out an undesired activity of the native protein. For example, The product from a gene comprising a dominant negative mutation of a receptor can be used to constitutively activate or suppress a signal transduction cascade, allowing examination of the phenotype and thus the trait(s) controlled by that receptor and pathway. Alternatively, the protein arising from the gene comprising a dominant-negative mutation can be an inactive enzyme still capable

30   of binding to the same substrate as the native protein and therefore competes with such native protein.

Products from genes comprising dominant-negative mutations can also act upon the native protein itself to prevent activity. For example, the native protein may be active only as a homo-multimer or as one subunit of a hetero-multimer. Incorporation of an inactive subunit into the multimer with native subunit(s) can inhibit activity.

5   Thus, gene function can be modulated in host cells of interest by insertion into these cells vector constructs comprising a gene comprising a dominant-negative mutation.

III.B.   Enhanced Expression

Enhanced expression of a gene of interest in a host cell can be accomplished by either (1) insertion of an exogenous gene; or (2) promoter modulation.

10   III.B.1. Insertion of an Exogenous Gene

Insertion of an expression construct encoding an exogenous gene can boost the number of gene copies expressed in a host cell.

Such expression constructs can comprise genes that either encode the native protein that is of interest or that encode a variant that exhibits enhanced activity as compared to

15   the native protein. Such genes encoding proteins of interest can be constructed from the sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto.

Such an exogenous gene can include either a constitutive promoter permitting expression in any cell in a host organism or a promoter that directs transcription only in particular cells or times during a host cell life cycle or in response to environmental stimuli.

20   III.B.2. Regulatory Sequence Modulation

The SDFs of Table 1, and fragments thereof, contain regulatory sequences that can be used to enhance expression of a gene of interest. For example, some of these sequences contain useful enhancer elements. In some cases, duplication of enhancer elements or insertion of exogenous enhancer elements will increase expression of a desired gene from a particular

25   promoter. As other examples, all ll promoters require binding of a regulatory protein to be activated, while some promoters may need a protein that signals a promoter binding protein to expose a polymerase binding site. In either case, over-production of such proteins can be used to enhance expression of a gene of interest by increasing the activation time of the promoter.

Such regulatory proteins are encoded by some of the sequences in TABLE 1,

30   fragments thereof, and substantially similar sequences thereto.

Coding sequences for these proteins can be constructed as described above.

IV. Gene Constructs and Vector Construction

To use isolated SDFs of the present invention or a combination of them or parts and/or mutants and/or fusions of said SDFs in the above techniques, recombinant DNA vectors which comprise said SDFs and are suitable for transformation of cells, such as plant cells, are usually prepared. The SDF construct can be made using standard recombinant DNA techniques (Sambrook et al. 1989) and can be introduced to the species of interest by *Agrobacterium*-mediated transformation or by other means of transformation (*e.g.*, particle gun bombardment) as referenced below.

The vector backbone can be any of those typical in the art such as plasmids, viruses, artificial chromosomes, BACs, YACs and PACs and vectors of the sort described by

(a) **BAC:** Shizuya et al., Proc. Natl. Acad. Sci. USA 89: 8794-8797 (1992); Hamilton et al., Proc. Natl. Acad. Sci. USA 93: 9975-9979 (1996);

(b) **YAC:** Burke et al., Science 236:806-812 (1987);.

(c) **PAC:** Sternberg N. et al., Proc Natl Acad Sci U S A. Jan;87(1):103-7 (1990);

(d) **Bacteria-Yeast Shuttle Vectors:** Bradshaw et al., Nucl Acids Res 23: 4850-4856 (1995);

(e) **Lambda Phage Vectors:** Replacement Vector, e.g., Frischauf et al., J. Mol Biol 170: 827-842 (1983); or Insertion vector, e.g., Huynh et al., In: Glover NM (ed) DNA Cloning: A practical Approach, Vol.1 Oxford: IRL Press (1985);

(f) **T-DNA gene fusion vectors :**Walden et al., Mol Cell Biol 1: 175-194 (1990); and

(g) **Plasmid vectors:** Sambrook et al., infra.

Typically, a vector will comprise the exogenous gene, which in its turn comprises an SDF of the present invention to be introduced into the genome of a host cell, and which gene may be an antisense construct, a ribozyme construct chimeraplast, or a coding sequence with any desired transcriptional and/or translational regulatory sequences, such as promoters, UTRs, and 3' end termination sequences. Vectors of the invention can also include origins of replication, scaffold attachment regions (SARs), markers, homologous sequences, introns, etc.

A DNA sequence coding for the desired polypeptide, for example a cDNA sequence encoding a full length protein, will preferably be combined with transcriptional and translational

initiation regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant.

For example, for over-expression, a plant promoter fragment may be employed that will direct transcription of the gene in all tissues of a regenerated plant.   Alternatively, the plant

5   promoter may direct transcription of an SDF of the invention in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters).

If proper polypeptide productionis desired, a polyadenylation region at the 3'-end of the coding region is typically included. The polyadenylation region can be derived from the natural

10   gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences from genes or SDF or the invention may comprise a marker gene that confers a selectable phenotype on plant cells. The vector can include promoter and coding sequence, for instance. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418,

15   bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or phosphinotricin.

IV.A.   Coding Sequences

Generally, the sequence in the transformation vector and to be introduced into the genome of the host cell does not need to be absolutely identical to an SDF of the present

20   invention. Also, it is not necessary for it to be full length, relative to either the primary transcription product or fully processed mRNA. Furthermore, the introduced sequence need not have the same intron or exon pattern as a native gene. Also, heterologous non-coding segments can be incorporated into the coding sequence without changing the desired amino acid sequence of the polypeptide to be produced.

25   IV.B.   Promoters

As explained above, introducing an exogenous SDF from the same species or an orthologous SDF from another species can modulate the expression of a native gene corresponding to that SDF of interest. Such an SDF construct can be under the control of either a constitutive promoter or a highly regulated inducible promoter (*e.g.*, a copper

30   inducible promoter). The promoter of interest can initially be either endogenous or heterologous to the species in question. When re-introduced into the genome of said species, such promoter becomes exogenous to said species. Over-expression of an SDF transgene can

lead to co-suppression of the homologous endogeneous sequence thereby creating some alterations in the phenotypes of the transformed species as demonstrated by similar analysis of the chalcone synthase gene (Napoli et al., *Plant Cell* 2:279 (1990) and van der Krol et al., *Plant Cell* 2:291 (1990)). If an SDF is found to encode a protein with desirable

5   characteristics, its over-production can be controlled so that its accumulation can be manipulated in an organ- or tissue-specific manner utilizing a promoter having such specificity.

Likewise, if the promoter of an SDF (or an SDF that includes a promoter) is found to be tissue-specific or developmentally regulated, such a promoter can be utilized to drive or

10   facilitate the transcription of a specific gene of interest (*e.g.*, seed storage protein or root-specific protein). Thus, the level of accumulation of a particular protein can be manipulated or its spatial localization in an organ- or tissue- specific manner can be altered.

IV. C   Signal Peptides

15   SDFs of the present invention containing signal peptides are indicated in Table 1. In some cases it may be desirable for the protein encoded by an introduced exogenous or orthologous SDF to be targeted (1) to a particular organelle intracellular compartment, (2) to interact with a particular molecule such as a membrane molecule or (3) for secretion outside of the cell harboring the introduced SDF. This will be accomplished using a signal peptide.

20   Signal peptides direct protein targeting, are involved in ligand-receptor interactions and act in cell to cell communication. Many proteins, especially soluble proteins, contain a signal peptide that targets the protein to one of several different intracellular compartments. In plants, these compartments include, but are not limited to, the endoplasmic reticulum (ER), mitochondria, plastids (such as chloroplasts), the vacuole, the Golgi apparatus, protein

25   storage vessicles (PSV) and, in general, membranes. Some signal peptide sequences are conserved, such as the Asn-Pro-Ile-Arg amino acid motif found in the N-terminal propeptide signal that targets proteins to the vacuole (Marty (1999) *The Plant Cell* 11: 587-599). Other signal peptides do not have a consensus sequence *per se*, but are largely composed of hydrophobic amino acids, such as those signal peptides targeting proteins to the ER (Vitale

30   and Denecke (1999) *The Plant Cell* 11: 615-628). Still others do not appear to contain either a consensus sequence or an identified common secondary sequence, for instance the chloroplast stromal targeting signal peptides (Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). Furthermore, some targeting peptides are bipartite, directing proteins first to an organelle and then to a membrane within the organelle (e.g. within the thylakoid lumen of the

chloroplast; see Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). In addition to the diversity in sequence and secondary structure, placement of the signal peptide is also varied. Proteins destined for the vacuole, for example, have targeting signal peptides found at the N-terminus, at the C-terminus and at a surface location in mature, folded proteins. Signal

5   peptides also serve as ligands for some receptors.

These characteristics of signal proteins can be used to more tightly control the phenotypic expression of introduced SDFs. In particular, associating the appropriate signal sequence with a specific SDF can allow sequestering of the protein in specific organelles (plastids, as an example), secretion outside of the cell, targeting interaction with particular

10   receptors, etc. Hence, the inclusion of signal proteins in constructs involving the SDFs of the invention increases the range of manipulation of SDF phenotypic expression. The nucleotide sequence of the signal peptide can be isolated from characterized genes using common molecular biological techniques or can be synthesized in vitro.

In addition, the native signal peptide sequences, both amino acid and nucleotide, described in Table 1 can be used to modulate polypeptide transport. Further variants of the native signal peptides described in Table 1 are contemplated. Insertions, deletions, or substitutions can be made. Such variants will retain at least one of the functions of the native signal peptide as well as exhibiting some degree of sequence identity to the native sequence.

Also, fragments of the signal peptides of the invention are useful and can be fused with other signal peptides of interest to modulate transport of a polypeptide.

V.   Transformation Techniques

15   A wide range of techniques for inserting exogenous polynucleotides are known for a number of host cells, including, without limitation, bacterial, yeast, mammalian, insect and plant cells.

Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. *See, e.g.* Weising et al., *Ann. Rev. Genet.*

20   22:421 (1988); and Christou, Euphytica, v. 85, n.1-3:13-27, (1995).

DNA constructs of the invention may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA constructs can be

25   introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and

introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria (McCormac et al., *Mol. Biotechnol.* 8:199 (1997); Hamilton, *Gene* 200:107 (1997)); Salomon et al. *EMBO J.* 3:141

5   (1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983).

Microinjection techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al. *EMBO J.* 3:2717 (1984). Electroporation techniques are described in Fromm et al. *Proc. Natl Acad. Sci. USA* 82:5824 (1985). Ballistic transformation

10   techniques are described in Klein et al. *Nature* 327:773 (1987). *Agrobacterium tumefaciens*-mediated transformation techniques, including disarming and use of binary or co-integrate vectors, are well described in the scientific literature. See, for example Hamilton, *CM.*, *Gene* 200:107 (1997); Müller et al. *Mol. Gen. Genet.* 207:171 (1987); Komari et al. *Plant J.* 10:165 (1996); Venkateswarlu et al. *Biotechnology* 9:1103 (1991) and Gleave, *AP.*, *Plant Mol.*

15   *Biol.* 20:1203 (1992); Graves and Goldman, *Plant Mol. Biol.* 7:34 (1986) and Gould et al., *Plant Physiology* 95:426 (1991).

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant that possesses the transformed genotype and thus the desired phenotype such as seedlessness. Such regeneration techniques rely on

20   manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture* in   Handbook of Plant Cell Culture," pp. 124-176, MacMillan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants, Plant Protoplasts,* pp. 21-73,

25   CRC Press, Boca Raton, 1988. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. *Ann. Rev. of Plant Phys.* 38:467 (1987). Regeneration of monocots (rice) is described by Hosoyama et al. (*Biosci. Biotechnol. Biochem.* 58:1500 (1994)) and by Ghosh et al. (*J. Biotechnol.* 32:1 (1994)). The nucleic acids of the invention can be used to confer desired traits on essentially any

30   plant.

Thus, the invention has use over a broad range of plants, including species from the genera *Anacardium, Arachis, Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Carthamus, Cocos, Coffea, Cucumis, Cucurbita, Daucus, Elaeis, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium,Lupinus,*

Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannesetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna, and, Zea.

5   One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

        The particular sequences of SDFs identified are provided in the attached TABLE 1.

10   One of ordinary skill in the art, having this data, can obtain cloned DNA fragments, synthetic DNA fragments or polypeptides constituting desired sequences by recombinant methodology known in the art or described herein.

EXAMPLES

        The invention is illustrated by way of the following examples. The invention is not

15   limited by these examples as the scope of the invention is defined solely by the claims following.

EXAMPLE 1: cDNA PREPARATION

        A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can be obtained by sequencing genomic DNA (gDNA) and/or cDNA

20   from corn plants grown from HYBRID SEED # 35A19, purchased from Pioneer Hi-Bred International, Inc., Supply Management, P.O. Box 256, Johnston, Iowa 50131-0256.

        A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can also be obtained by sequencing genomic DNA from *Arabidopsis thaliana*, Wassilewskija ecotype or by sequencing cDNA obtained from mRNA

25   from such plants as described below. This is a true breeding strain. Seeds of the plant are available from the Arabidopsis Biological Resource Center at the Ohio State University, under the accession number CS2360. Seeds of this plant were deposited under the terms and conditions of the Budapest Treaty at the American Type Culture Collection, Manassas, VA on August 31, 1999, and were assigned ATCC No. PTA-595.

30   Other methods for cloning full-length cDNA are described, for example, by Seki et al., *Plant Journal* 15:707-720 (1998) High-efficiency cloning of Arabidopsis full-length cDNA by biotinylated Cap trapper"; Maruyama et al., *Gene* 138:171 (1994) Oligo-capping a

simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides";
and WO 96/34981.

Tissues were, or each organ was, individually pulverized and frozen in liquid
nitrogen. Next, the samples were homogenized in the presence of detergents and then
5   centrifuged. The debris and nuclei were removed from the sample and more detergents were
added to the sample. The sample was centrifuged and the debris was removed. Then the
sample was applied to a 2M sucrose cushion to isolate polysomes. The RNA was isolated by
treatment with detergents and proteinase K followed by ethanol precipitation and
centrifugation. The polysomal RNA from the different tissues was pooled according to the
10   following mass ratios: 15/15/1 for male inflorescences, female inflorescences and root,
respectively. The pooled material was then used for cDNA synthesis by the methods
described below.

Starting material for cDNA synthesis for the exemplary corn cDNA clones
with sequences presented in TABLE 1 was poly(A)-containing polysomal mRNAs from
15   inflorescences and root tissues of corn plants grown from HYBRID SEED # 35A19. Male
inflorescences and female (pre-and post-fertilization) inflorescences were isolated at various
stages of development. Selection for poly(A) containing polysomal RNA was done using
oligo d(T) cellulose columns, as described by Cox and Goldberg, Plant Molecular Biology:
A Practical Approach", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The quality and the
20   integrity of the polyA+ RNAs were evaluated.

Starting material for cDNA synthesis for the exemplary *Arabidopsis* cDNA
clones with sequences presented in TABLE 1 was polysomal RNA isolated from the top-
most inflorescence tissues of *Arabidopsis thaliana* Wassilewskija (Ws.) and from roots of
25   *Arabidopsis thaliana* Landsberg erecta (L. er.), also obtained from the Arabidopsis
Biological Resource Center. Nine parts inflorescence to every part root was used, as
measured by wet mass. Tissue was pulverized and exposed to liquid nitrogen. Next, the
sample was homogenized in the presence of detergents and then centrifuged. The debris and
nuclei were removed from the sample and more detergents were added to the sample. The
30   sample was centrifuged and the debris was removed and the sample was applied to a 2M
sucrose cushion to isolate polysomal RNA. Cox et al., Plant Molecular Biology: A Practical
Approach", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The polysomal RNA was used
for cDNA synthesis by the methods described below. Polysomal mRNA was then isolated as
described above for corn cDNA. The quality of the RNA was assessed electrophoretically.

Following preparation of the mRNAs from various tissues as described above, selection of mRNA with intact 5' ends and specific attachment of an oligonucleotide tag to the 5' end of such mRNA was performed using either a chemical or enzymatic approach. Both techniques take advantage of the presence of the cap" structure, which characterizes the 5' end of most

5   intact mRNAs and which comprises a guanosine generally methylated once, at the 7 position.

The chemical modification approach involves the optional elimination of the 2', 3'-cis diol of the 3' terminal ribose, the oxidation of the 2', 3'-cis diol of the ribose linked to the cap of the 5' ends of the mRNAs into a dialdehyde, and the coupling of the such obtained dialdehyde to a derivatized oligonucleotide tag. Further detail regarding the chemical approaches for

10   obtaining mRNAs having intact 5' ends are disclosed in International Application No. WO96/34981 published November 7, 1996.

The enzymatic approach for ligating the oligonucleotide tag to the intact 5' ends of mRNAs involves the removal of the phosphate groups present on the 5' ends of uncapped incomplete mRNAs, the subsequent decapping of mRNAs having intact 5' ends and the ligation

15   of the phosphate present at the 5' end of the decapped mRNA to an oligonucleotide tag. Further detail regarding the enzymatic approaches for obtaining mRNAs having intact 5' ends are disclosed in Dumas Milne Edwards J.B. (Doctoral Thesis of Paris VI University, Le clonage des ADNc complets: difficultés et perspectives nouvelles. Apports pour l'étude de la régulation de l'expression de la tryptophane hydroxylase de rat, 20 Dec. 1993), EPO 625572 and Kato *et al.*,

20   *Gene* 150:243-250 (1994).

In both the chemical and the enzymatic approach, the oligonucleotide tag has a restriction enzyme site (e.g. an EcoRI site) therein to facilitate later cloning procedures. Following attachment of the oligonucleotide tag to the mRNA, the integrity of the mRNA is examined by performing a Northern blot using a probe complementary to the oligonucleotide

25   tag.

For the mRNAs joined to oligonucleotide tags using either the chemical or the enzymatic method, first strand cDNA synthesis is performed using an oligo-dT primer with reverse transcriptase. This oligo-dT primer can contain an internal tag of at least 4 nucleotides, which can be different from one mRNA preparation to another. Methylated dCTP is used for cDNA

30   first strand synthesis to protect the internal EcoRI sites from digestion during subsequent steps. The first strand cDNA is precipitated using isopropanol after removal of RNA by alkaline hydrolysis to eliminate residual primers.

Second strand cDNA synthesis is conducted using a DNA polymerase, such as Klenow fragment and a primer corresponding to the 5' end of the ligated oligonucleotide. The primer is

typically 20-25 bases in length. Methylated dCTP is used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

Following second strand synthesis, the full-length cDNAs are cloned into a phagemid vector, such as pBlueScript™ (Stratagene). The ends of the full-length cDNAs are blunted with

5   T4 DNA polymerase (Biolabs) and the cDNA is digested with EcoRI. Since methylated dCTP is used during cDNA synthesis, the EcoRI site present in the tag is the only hemi-methylated site; hence the only site susceptible to EcoRI digestion. In some instances, to facilitate subcloning, an Hind III adapter is added to the 3' end of full-length cDNAs.

The full-length cDNAs are then size fractionated using either exclusion chromatography

10   (AcA, Biosepra) or electrophoretic separation which yields 3 to 6 different fractions. The full-length cDNAs are then directionally cloned either into pBlueScript™ using either the EcoRI and SmaI restriction sites or, when the Hind III adapter is present in the full-length cDNAs, the EcoRI and Hind III restriction sites. The ligation mixture is transformed, preferably by electroporation, into bacteria, which are then propagated under appropriate antibiotic selection.

15   Clones containing the oligonucleotide tag attached to full-length cDNAs are selected as follows.

The plasmid cDNA libraries made as described above are purified (e.g. by a column available from Qiagen). A positive selection of the tagged clones is performed as follows. Briefly, in this selection procedure, the plasmid DNA is converted to single stranded DNA using

20   phage F1 gene II endonuclease in combination with an exonuclease (Chang et al., *Gene* 127:95 (1993)) such as exonuclease III or T7 gene 6 exonuclease. The resulting single stranded DNA is then purified using paramagnetic beads as described by Fry et al., *Biotechniques* 13: 124 (1992). Here the single stranded DNA is hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide tag. Preferably, the primer has a

25   length of 20-25 bases. Clones including a sequence complementary to the biotinylated oligonucleotide are selected by incubation with streptavidin coated magnetic beads followed by magnetic capture. After capture of the positive clones, the plasmid DNA is released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as ThermoSequenase™ (obtained from Amersham Pharmacia Biotech). Alternatively, protocols

30   such as the Gene Trapper™ kit (Gibco BRL) can be used. The double stranded DNA is then transformed, preferably by electroporation, into bacteria. The percentage of positive clones having the 5' tag oligonucleotide is typically estimated to be between 90 and 98% from dot blot analysis.

Following transformation, the libraries are ordered in microtiter plates and sequenced. The *Arabidopsis* library was deposited at the American Type Culture Collection on January 7, 2000 as *E-coli* liba 010600" under the accession number **PTA-1161.**

EXAMPLE 2: SOUTHERN HYBRIDIZATIONS

5   The SDFs of the invention can be used in Southern hybridizations as described above. The following describes extraction of DNA from nuclei of plant cells, digestion of the nuclear DNA and separation by length, transfer of the separated fragments to membranes, preparation of probes for hybridization, hybridization and detection of the hybridized probe.

The procedures described herein can be used to isolate related polynucleotides or for 10   diagnostic purposes. Moderate stringency hybridization conditions, as defined above, are described in the present example. These conditions result in detection of hybridization between sequences having at least 70% sequence identity. As described above, the hybridization and wash conditions can be changed to reflect the desired percenatge of sequence identity between probe and target sequences that can be detected.

15   In the following procedure, a probe for hybridization is produced from two PCR reactions using two primers from genomic sequence of *Arabidopsis thaliana*. As described above, the particular template for generating the probe can be any desired template.

The first PCR product is assessed to validate the size of the primer to assure it is of the expected size. Then the product of the first PCR is used as a template, with the same pair 20   of primers used in the first PCR, in a second PCR that produces a labeled product used as the probe.

Fragments detected by hybridization, or other bands of interest, can be isolated from gels used to separate genomic DNA fragments by known methods for further purification and/or characterization.

25   **Buffers for nuclear DNA extraction**

1.   10X HB

	1000 ml	
40 mM spermidine	10.2 g	Spermine (Sigma S-2876) and spermidine (Sigma S-2501)
10 mM spermine	3.5 g	Stabilize chromatin and the nuclear membrane

0.1 M EDTA (disodium)	37.2 g	EDTA inhibits nuclease
0.1 M Tris	12.1 g	Buffer
0.8 M KCl	59.6 g	Adjusts ionic strength for stability of nuclei

Adjust pH to 9.5 with 10 N NaOH. It appears that there is a nuclease present in leaves. Use of pH 9.5 appears to inactivate this nuclease.

2. 2 M sucrose   (684 g per 1000 ml)

Heat about half the final volume of water to about 50°C. Add the sucrose slowly then bring the mixture to close to final volume; stir constantly until it has dissolved. Bring the solution to volume.

3. Sarkosyl solution (lyses nuclear membranes)

	1000 ml
N-lauroyl sarcosine (Sarkosyl)	20.0 g
0.1 M Tris	12.1 g
0.04 M EDTA (Disodium)	14.9 g

Adjust the pH to 9.5 after all the components are dissolved and bring up to the proper volume.

4. 20% Triton X-100

80 ml Triton X-100

320 ml 1xHB (w/o β-ME and PMSF)

Prepare in advance; Triton takes some time to dissolve

A. Procedure

1. Prepare 1X H" buffer (keep ice-cold during use)

	1000 ml
10X HB	100 ml
2 M sucrose	250 ml a non-ionic osmoticum
Water	634 ml

5 **Added just before use:**

100 mM PMSF*	10 ml a protease inhibitor; protects nuclear membrane proteins
ß-mercaptoethanol	1 ml inactivates nuclease by reducing disulfide bonds

10 *100 mM PMSF

(phenyl methyl sulfonyl fluoride, Sigma P-7626)

(add 0.0875 g to 5 ml 100% ethanol)

2. Homogenize the tissue in a blender (use 300-400 ml of 1xHB per blender). Be sure that you use 5-10 ml of HB buffer per gram of tissue. Blenders generate heat so be

15 sure to keep the homogenate cold. It is necessary to put the blenders in ice periodically.

3. Add the 20% Triton X-100 (25 ml per liter of homogenate) and gently stir on ice for 20 min. This lyses plastid, but not nuclear, membranes.

4. Filter the tissue suspension through several nylon filters into an ice-cold beaker. The

20 first filtration is through a 250-micron membrane; the second is through an 85-micron membrane; the third is through a 50-micron membrane; and the fourth is through a 20-micron membrane. Use a large funnel to hold the filters. Filtration can be sped up by gently squeezing the liquid through the filters.

5. Centrifuge the filtrate at 1200 x g for 20 min. at 4°C to pellet the nuclei.

6.   Discard the dark green supernatant. The pellet will have several layers to it. One is starch; it is white and gritty. The nuclei are gray and soft. In the early steps, there may be a dark green and somewhat viscous layer of chloroplasts.

Wash the pellets in about 25 ml cold H buffer (with Triton X-100) and resuspend by

5   swirling gently and pipetting. After the pellets are resuspended.

Pellet the nuclei again at 1200 - 1300 x g. Discard the supernatant.

Repeat the wash 3-4 times until the supernatant has changed from a dark green to a pale green. This usually happens after 3 or 4 resuspensions. At this point, the pellet is typically grayish white and very slippery. The Triton X-100 in these repeated steps

10   helps to destroy the chloroplasts and mitochondria that contaminate the prep.

Resuspend the nuclei for a final time in a total of 15 ml of H buffer and transfer the suspension to a sterile 125 ml Erlenmeyer flask.

7.   Add 15 ml, dropwise, cold 2% Sarkosyl, 0.1 M Tris, 0.04 M EDTA solution (pH 9.5) while swirling gently. This lyses the nuclei. The solution will become very viscous.

15   8.   Add 30 grams of CsCl and gently swirl at room temperature until the CsCl is in solution. The mixture will be gray, white and viscous.

9.   Centrifuge the solution at 11,400 x g at 4°C for at least 30 min. The longer this spin is, the firmer the protein pellicle.

20   10.   The result is typically a clear green supernatant over a white pellet, and (perhaps) under a protein pellicle. Carefully remove the solution under the protein pellicle and above the pellet. Determine the density of the solution by weighing 1 ml of solution and add CsCl if necessary to bring to 1.57 g/ml. The solution contains dissolved solids (sucrose etc) and the refractive index alone will not be an accurate guide to

25   CsCl concentration.

11. Add 20 µl of 10 mg/ml EtBr per ml of solution.

12. Centrifuge at 184,000 x g for 16 to 20 hours in a fixed-angle rotor.

13. Remove the dark red supernatant that is at the top of the tube with a plastic transfer pipette and discard. Carefully remove the DNA band with another transfer pipette. The DNA band is usually visible in room light; otherwise, use a long wave UV light to locate the band.

14. Extract the ethidium bromide with isopropanol saturated with water and salt. Once the solution is clear, extract at least two more times to ensure that all of the EtBr is gone. Be very gentle, as it is very easy to shear the DNA at this step. This extraction may take a while because the DNA solution tends to be very viscous. If the solution is too viscous, dilute it with TE.

15. Dialyze the DNA for at least two days against several changes (at least three times) of TE (10 mM Tris, 1mM EDTA, pH 8) to remove the cesium chloride.

16. Remove the dialyzed DNA from the tubing. If the dialyzed DNA solution contains a lot of debris, centrifuge the DNA solution at least at 2500 x g for 10 min. and carefully transfer the clear supernatant to a new tube. Read the A260 concentration of the DNA.

17. Assess the quality of the DNA by agarose gel electrophoresis (1% agarose gel) of the DNA. Load 50 ng and 100 ng (based on the OD reading) and compare it with known and good quality DNA. Undigested lambda DNA and a lambda-HindIII-digested DNA are good molecular weight makers.

Protocol for Digestion of Genomic DNA

Protocol:

1. The relative amounts of DNA for different crop plants that provide approximately a balanced number of genome equivalent is given in Table 3. Note that due to the size

of the wheat genome, wheat DNA will be underrepresented. Lambda DNA provides a useful control for complete digestion.

2. Precipitate the DNA by adding 3 volumes of 100% ethanol. Incubate at -20°C for at least two hours. Yeast DNA can be purchased and made up at the necessary

5   concentration, therefore no precipitation is necessary for yeast DNA.

3. Centrifuge the solution at 11,400 x g for 20 min. Decant the ethanol carefully (be careful not to disturb the pellet). Be sure that the residual ethanol is completely removed either by vacuum desiccation or by carefully wiping the sides of the tubes with a clean tissue.

10   4. Resuspend the pellet in an appropriate volume of water. Be sure the pellet is fully resuspended before proceeding to the next step. This may take about 30 min.

5. Add the appropriate volume of 10X reaction buffer provided by the manufacturer of the restriction enzyme to the resuspended DNA followed by the appropriate volume of enzymes. Be sure to mix it properly by slowly swirling the tubes.

15   6. Set-up the lambda digestion-control for each DNA that you are digesting.

7. Incubate both the experimental and lambda digests overnight at 37°C. Spin down condensation in a microfuge before proceeding.

8. After digestion, add 2 µl of loading dye (typically 0.25% bromophenol blue, 0.25% xylene cyanol in 15% Ficoll or 30% glycerol) to the lambda-control digests and load

20   in 1% TPE-agarose gel (TPE is 90 mM Tris-phosphate, 2 mM EDTA, pH 8). If the lambda DNA in the lambda control digests are completely digested, proceed with the precipitation of the genomic DNA in the digests.

9. Precipitate the digested DNA by adding 3 volumes of 100% ethanol and incubating in -20°C for at least 2 hours (preferably overnight).

EXCEPTION: *Arabidopsis* and yeast DNA are digested in an appropriate volume; they don't have to be precipitated.

10.   Resuspend the DNA in an appropriate volume of TE (e.g., 22 µl x 50 blots = 1100 µl) and an appropriate volume of 10X loading dye (e.g., 2.4 µl x 50 blots = 120 µl). Be careful in pipetting the loading dye - it is viscous. Be sure you are pipetting the correct volume.

Table 3

Some guide points in digesting genomic DNA.

Species	Genome Size	Size Relative to Arabidopsis	Genome Equivalent to 2 µg Arabidopsis DNA	Amount of DNA per blot
Arabidopsis	120 Mb	1X	1X	2 µg
Brassica	1,100 Mb	9.2X	0.54X	10 µg
Corn	2,800 Mb	23.3X	0.43X	20 µg
Cotton	2,300 Mb	19.2X	0.52X	20 µg
Oat	11,300 Mb	94X	0.11X	20 µg
Rice	400 Mb	3.3X	0.75X	5 µg
Soybean	1,100 Mb	9.2X	0.54X	10 µg
Sugarbeet	758 Mb	6.3X	0.8X	10 µg
Sweetclover	1,100 Mb	9.2X	0.54X	10 µg
Wheat	16,000 Mb	133X	0.08X	20 µg
Yeast	15 Mb	0.12X	1X	0.25 µg

Protocol for Southern Blot Analysis

The digested DNA samples are electrophoresed in 1% agarose gels in 1x TPE buffer. Low voltage; overnight separations are preferred. The gels are stained with EtBr and photographed.

1. For blotting the gels, first incubate the gel in 0.25 N HCl (with gentle shaking) for about 15 min.

2. Then briefly rinse with water. The DNA is denatured by 2 incubations. Incubate (with shaking) in 0.5 M NaOH in 1.5 M NaCl for 15 min.

5 3. The gel is then briefly rinsed in water and neutralized by incubating twice (with shaking) in 1.5 M Tris pH 7.5 in 1.5 M NaCl for 15 min.

4. A nylon membrane is prepared by soaking it in water for at least 5 min, then in 6X SSC for at least 15 min. before use. (20x SSC is 175.3 g NaCl, 88.2 g sodium citrate per liter, adjusted to pH 7.0.)

10 5. The nylon membrane is placed on top of the gel and all bubbles in between are removed. The DNA is blotted from the gel to the membrane using an absorbent medium, such as paper toweling and 6x SCC buffer. After the transfer, the membrane may be lightly brushed with a gloved hand to remove any agarose sticking to the surface.

15 6. The DNA is then fixed to the membrane by UV crosslinking and baking at 80°C. The membrane is stored at 4°C until use.

B. Protocol for PCR Amplification of Genomic Fragments in Arabidopsis

Amplification procedures:

1. Mix the following in a 0.20 ml PCR tube or 96-well PCR plate:

Volume	Stock	Final Amount or Conc.
0.5 μl	~ 10 ng/μl genomic DNA[1]	5 ng
2.5 μl	**10X PCR buffer**	20 mM Tris, 50 mM KCl

---

[1] Arabidopsis DNA is used in the present experiment, but the procedure is a general one.

0.75 µl	50 mM MgCl₂	1.5 mM
1 µl	10 pmol/µl Primer 1 (Forward)	10 pmol
1 µl	10 pmol/µl Primer 2 (Reverse)	10 pmol
0.5 µl	5 mM dNTPs	0.1 mM
0.1 µl	5 units/µl Platinum Taq™ (Life Technologies, Gaithersburg, MD) DNA Polymerase	1 units
(to 25 µl)	**Water**	

2.   The template DNA is amplified using a Perkin Elmer 9700 PCR machine:

1)   94°C for 10 min. followed by

2) 5 cycles:	3) 5 cycles:	4) 25 cycles:
94 °C - 30 sec 62 °C - 30 sec 72 °C - 3 min	94 °C - 30 sec 58 °C - 30 sec 72 °C - 3 min	94 °C - 30 sec 53 °C - 30 sec 72 °C - 3 min

5)   72°C for 7 min. Then the reactions are stopped by chilling to 4°C.

The procedure can be adapted to a multi-well format if necessary.

Quantification and Dilution of PCR Products:

1.   The product of the PCR is analyzed by electrophoresis in a 1% agarose gel. A linearized plasmid DNA can be used as a quantification standard (usually at 50, 100,

200, and 400 ng). These will be used as references to approximate the amount of PCR products. HindIII-digested Lambda DNA is useful as a molecular weight marker. The gel can be run fairly quickly; e.g., at 100 volts. The standard gel is examined to determine that the size of the PCR products is consistent with the expected size and if there are significant extra bands or smeary products in the PCR reactions.

2.   The amounts of PCR products can be estimated on the basis of the plasmid standard.

3.   For the small number of reactions that produce extraneous bands, a small amount of DNA from bands with the correct size can be isolated by dipping a sterile 10-μl tip into the band while viewing though a UV Transilluminator. The small amount of agarose gel (with the DNA fragment) is used in the labeling reaction.

C.   Protocol for PCR-DIG-Labeling of DNA

<u>Solutions</u>:

Reagents in PCR reactions (diluted PCR products, 10X PCR Buffer, 50 mM $MgCl_2$, 5 U/μl Platinum Taq Polymerase, and the primers)

10X dNTP + DIG-11-dUTP **[1:5]**:   (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.65 mM dTTP, 0.35 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP **[1:10]**:   (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.81 mM dTTP, 0.19 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP **[1:15]**:   (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.875 mM dTTP, 0.125 mM DIG-11-dUTP)

TE buffer (10 mM Tris, 1 mM EDTA, pH 8)

Maleate buffer: In 700 ml of deionized distilled water, dissolve 11.61 g maleic acid and 8.77 g NaCl. Add NaOH to adjust the pH to 7.5. Bring the volume to 1 L. Stir for 15 min. and sterilize.

10% blocking solution: In 80 ml deionized distilled water, dissolve 1.16g maleic acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent powder (Boehringer Mannheim, Indianapolis, IN, Cat. no. 1096176). Heat to 60°C while stirring to dissolve the powder. Adjust the volume to 100 ml with water. Stir and sterilize.

1% blocking solution: Dilute the 10% stock to 1% using the maleate buffer.

Buffer 3 (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$, pH9.5). Prepared from autoclaved solutions of 1M Tris pH 9.5, 5 M NaCl, and 1 M $MgCl_2$ in autoclaved distilled water.

Procedure:

1.   PCR reactions are performed in 25 µl volumes containing:

PCR buffer	1X
$MgCl_2$	1.5 mM
10X dNTP + DIG-11-dUTP	1X (please see the note below)
Platinum Taq™ Polymerase	1 unit
10 pg probe DNA	
10 pmol primer 1	

Note:	Use for:
10X dNTP + DIG-11-dUTP (1:5)	< 1 kb
10X dNTP + DIG-11-dUTP (1:10)	1 kb to 1.8 kb
10X dNTP + DIG-11-dUTP (1:15)	> 1.8 kb

2.   The PCR reaction uses the following amplification cycles:

1) $94°C$ for 10 min.

2) 5 cycles:	3) 5 cycles:	4) 25 cycles:
$95°C$ - 30 sec $61°C$ - 1 min $73°C$ - 5 min	$95°C$ - 30 sec $59°C$ - 1 min $75°C$ - 5 min	$95°C$ - 30 sec $51°C$ - 1 min $73°C$ - 5 min

5) $72°C$ for 8 min. The reactions are terminated by chilling to $4°C$ (hold).

3.   The products are analyzed by electrophoresis- in a 1% agarose gel, comparing to an aliquot of the unlabelled probe starting material.

4.   The amount of DIG-labeled probe is determined as follows:

Make serial dilutions of the diluted control DNA in dilution buffer (TE: 10 mM Tris and 1 mM EDTA, pH 8) as shown in the following table:

DIG-labeled control DNA starting conc.	Stepwise Dilution	Final Conc. (Dilution Name)
5 ng/μl	1 μl in 49 μl TE	100 pg/μl (A)
100 pg/μl (A)	25 μl in 25 μl TE	50 pg/μl (B)
50 pg/μl (B)	25 μl in 25 μl TE	25 pg/μl (C)
25 pg/μl (C)	20 μl in 30 μl TE	10 pg/μl (D)

a.   Serial deletions of a DIG-labeled standard DNA ranging from 100 pg to 10 pg are spotted onto a positively charged nylon membrane, marking the membrane lightly with a pencil to identify each dilution.

b.   Serial dilutions (e.g., 1:50, 1:2500, 1:10,000) of the newly labeled DNA probe are spotted.

c.   The membrane is fixed by UV crosslinking.

d.   The membrane is wetted with a small amount of maleate buffer and then incubated in 1% blocking solution for 15 min at room temp.

e.   The labeled DNA is then detected using alkaline phosphatase conjugated anti-DIG antibody (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274) and an NBT substrate according to the manufacture's instruction.

f.   Spot intensities of the control and experimental dilutions are then compared to estimate the concentration of the PCR-DIG-labeled probe.

D. **Prehybridization and Hybridization of Southern Blots**

Solutions:

    100% Formamide   purchased from Gibco

    20X SSC   (1X = 0.15 M NaCl, 0.015 M Na_3citrate)

5   per L: 175 g NaCl

                                        87.5 g Na_3citrate·$2H_2O$

    20% Sarkosyl (N-lauroyl-sarcosine)

    20% SDS (sodium dodecyl sulphate)

    10% Blocking Reagent: In 80 ml deionized distilled water, dissolve 1.16 g maleic

10   acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent

                powder. Heat to 60°C while stirring to dissolve the powder. Adjust the volume

                to 100 ml with water. Stir and sterilize.

Prehybridization Mix:

Final Concentration	Components	Volume (per 100 ml)	Stock
50%	Formamide	50 ml	100%
5X	SSC	25 ml	20X
0.1%	Sarkosyl	0.5 ml	20%
0.02%	SDS	0.1 ml	20%
2%	Blocking Reagent	20 ml	10%
	Water	4.4 ml	

General Procedures:

15   1.   Place the blot in a heat-sealable plastic bag and add an appropriate volume of

                prehybridization solution (30 ml/100cm^2) at room temperature. Seal the bag with a

                heat sealer, avoiding bubbles as much as possible. Lay down the bags in a large

                plastic tray (one tray can accommodate at least 4–5 bags). Ensure that the bags are

lying flat in the tray so that the prehybridization solution is evenly distributed throughout the bag. Incubate the blot for at least 2 hours with gentle agitation using a waver shaker.

2. Denature DIG-labeled DNA probe by incubating for 10 min. at 98°C using the PCR
5   machine and immediately cool it to 4°C.

3. Add probe to prehybridization solution (25 ng/ml; 30 ml = 750 ng total probe) and mix well but avoid foaming. Bubbles may lead to background.

4. Pour off the prehybridization solution from the hybridization bags and add new prehybridization and probe solution mixture to the bags containing the membrane.

10 5. Incubate with gentle agitation for at least 16 hours.

6. Proceed to medium stringency post-hybridization wash:

Three times for 20 min. each with gentle agitation using 1X SSC, 1% SDS at 60°C.

All wash solutions must be prewarmed to 60°C. Use about 100 ml of wash solution per membrane.

15   To avoid background keep the membranes fully submerged to avoid drying in spots; agitate sufficiently to avoid having membranes stick to one another.

7. After the wash, proceed to immunological detection and CSPD development.

E.   Procedure for Immunological Detection with CSPD

Solutions:

20 Buffer 1:   Maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl; adjusted to pH 7.5 with NaoH)

Washing buffer: Maleic acid buffer with 0.3% (v/v) Tween 20.

Blocking stock solution   10% blocking reagent in buffer 1. Dissolve (10X

concentration): blocking reagent powder (Boehringer

Mannheim, Indianapolis, IN, cat. no. 1096176) by

constantly stirring on a 65°C heating block or heat in a

5   microwave, autoclave and store at 4°C.

Buffer 2

(1X blocking solution):   Dilute the stock solution 1:10 in Buffer 1.

Detection buffer: 0.1 M Tris, 0.1 M NaCl, pH 9.5

Procedure:

10   1.   After the post-hybridization wash the blots are briefly rinsed (1-5 min.) in the maleate
           washing buffer with gentle shaking.

     2.   Then the membranes are incubated for 30 min. in Buffer 2 with gentle shaking.

     3.   Anti-DIG-AP conjugate (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274)
           at 75 mU/ml (1:10,000) in Buffer 2 is used for detection. 75 ml of solution can be
15   used for 3 blots.

     4.   The membrane is incubated for 30 min. in the antibody solution with gentle shaking.

     5.   The membrane are washed twice in washing buffer with gentle shaking. About 250
           mls is used per wash for 3 blots.

     6.   The blots are equilibrated for 2–5 min in 60 ml detection buffer.

20   7.   Dilute CSPD (1:200) in detection buffer. (This can be prepared ahead of time and
           stored in the dark at 4°C).

           The following steps must be done individually. Bags (one for detection and one for
           exposure) are generally cut and ready before doing the following steps.

8. The blot is carefully removed from the detection buffer and excess liquid removed without drying the membrane. The blot is immediately placed in a bag and 1.5 ml of CSPD solution is added. The CSPD solution can be spread over the membrane. Bubbles present at the edge and on the surface of the blot are typically removed by gentle rubbing. The membrane is incubated for 5 min. in CSPD solution.

9. Excess liquid is removed and the membrane is blotted briefly (DNA side up) on Whatman 3MM paper. Do not let the membrane dry completely.

10. Seal the damp membrane in a hybridization bag and incubate for 10 min at 37°C to enhance the luminescent reaction.

11. Expose for 2 hours at room temperature to X-ray film. Multiple exposures can be taken. Luminescence continues for at least 24 hours and signal intensity increases during the first hours.

Example 3: Transformation of Carrot Cells

Transformation of plant cells can be accomplished by a number of methods, as described above. Similarly, a number of plant genera can be regenerated from tissue culture following transformation. Transformation and regeneration of carrot cells as described herein is illustrative.

Single cell suspension cultures of carrot (*Daucus carota*) cells are established from hypocotyls of cultivar Early Nantes in B_5 growth medium (O.L. Gamborg et al., *Plant Physiol.* 45:372 (1970)) plus 2,4-D and 15 mM $CaCl_2$ (B_5 -44 medium) by methods known in the art. The suspension cultures are subcultured by adding 10 ml of the suspension culture to 40 ml of B_5-44 medium in 250 ml flasks every 7 days and are maintained in a shaker at 150 rpm at 27 °C in the dark.

The suspension culture cells are transformed with exogenous DNA as described by Z. Chen et al. *Plant Mol. Bio.* 36:163 (1998). Briefly, 4-days post-subculture cells are incubated with cell wall digestion solution containing 0.4 M sorbitol, 2% driselase, 5mM MES (2-[N-Morpholino] ethanesulfonic acid) pH 5.0 for 5 hours. The digested cells are pelleted gently at 60 xg for 5 min. and washed twice in W5 solution containing 154 mM NaCl, 5 mM KCl, 125 mM $CaCl_2$ and 5mM glucose, pH 6.0. The protoplasts are suspended in MC solution

containing 5 mM MES, 20 mM $CaCl_2$, 0.5 M mannitol, pH 5.7 and the protoplast density is adjusted to about 4 x 10^6 protoplasts per ml.

15-60 µg of plasmid DNA is mixed with 0.9 ml of protoplasts. The resulting suspension is mixed with 40% polyethylene glycol (MW 8000, PEG 8000), by gentle

5   inversion a few times at room temperature for 5 to 25 min. Protoplast culture medium known in the art is added into the PEG-DNA-protoplast mixture. Protoplasts are incubated in the culture medium for 24 hour to 5 days and cell extracts can be used for assay of transient expression of the introduced gene. Alternatively, transformed cells can be used to produce transgenic callus, which in turn can be used to produce transgenic plants, by methods known

10   in the art. See, for example, Nomura and Komamine, *Plt. Phys.* 79:988-991 (1985), *Identification and Isolation of Single Cells that Produce Somatic Embryos in Carrot Suspension Cultures.*

An additional deposit, PTA-1411, of an *E. coli* Library, *E. coli*LibA021800, was made at the American Type Culture Collection in Manassas, Virginia, USA on February 22,

15   2000 to meet the requirements of Budapest Treaty for the international recognition of the deposit of microorganisms. This deposit was assigned ATCC accession no. PTA-1411.

The invention being thus described, it will be apparent to one of ordinary skill in the art that various modifications of the materials and methods for practicing the invention can be made. Such modifications are to be considered within the scope of the invention as defined

20   by the following claims.

Each of the references from the patent and periodical literature cited herein is hereby expressly incorporated in its entirety by such citation.

Table 1

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	Intr	58662	58609	–	23
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15	Intr	59209	58994	–	23
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	Intr	59829	59763	–	23
	Intr	60166	60033	–	23
	Intr	60518	60419	–	23
20	Intr	61102	60621	–	23
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	Intr	80677	80845	+	28
55	Intr	80937	81030	+	28
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        Intr   11949   12027      +       4
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        Intr   12799   12846      +       4
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20      Intr   15203   15427      +       4
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30      Intr   23555   23709      +       6
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45      Intr   41571   41263      -      19
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60      Intr   55571   55416      -      22
        Intr   55793   55659      -      22
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	Intr	56045	55880	-	22
	Intr	56277	56199	-	22
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	Intr	56793	56741	-	22
5	Intr	57090	56910	-	22
	Init	57432	57268	-	22

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10	Init	62002	62015	+	24
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	Intr	75381	75104	-	31
	Intr	75745	75474	-	31
30	Intr	77420	76915	-	31
	Intr	78279	78094	-	31
	Intr	78637	78360	-	31
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	Intr	75381	75104	-	31
	Intr	75745	75474	-	31
	Intr	77420	76915	-	31
	Intr	78279	78094	-	31
45	Intr	78637	78360	-	31
	Init	79282	79063	-	31

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	Init	82947	82729	-	34

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	Term	94687	94667	-	40
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60	Intr	95457	95344	-	40
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					102
	Intr	95819	95748	−	40
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	Intr	96125	96039	−	40
	Intr	96311	96210	−	40
5	Intr	96440	96403	−	40
	Intr	96636	96534	−	40
	Intr	96842	96720	−	40
	Intr	96993	96917	−	40
	Intr	97339	97298	−	40
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	Intr	95457	95344	−	40
	Intr	95662	95544	−	40
20	Intr	95819	95748	−	40
	Intr	95952	95884	−	40
	Intr	96125	96039	−	40
	Intr	96311	96210	−	40
	Intr	96440	96403	−	40
25	Intr	96636	96534	−	40
	Intr	96842	96720	−	40
	Intr	96993	96917	−	40
	Intr	97339	97298	−	40
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	Intr	96125	96039	−	40
	Intr	96311	96210	−	40
	Intr	96440	96403	−	40
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50					
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55	Intr	112720	112761	+	45
	Intr	112852	112935	+	45
	Intr	113003	113090	+	45
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60	Term	113440	113605	+	45

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15  >6136349          /12983

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         Term  24730   24810     +     11

20  >6136349          /125682

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         Intr  48459   48754     +     23
         Intr  48903   48996     +     23
25       Term  49121   49293     +     23

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         Term  59531   58419     -     27
30       Intr  60237   60182     -     27
         Init  60662   60434     -     27

    >6136349          /158702

35       Term 109616  109467     -     39
         Intr 109838  109788     -     39
         Intr 110002  109924     -     39
         Intr 110469  110129     -     39
         Intr 110774  110715     -     39
40       Intr 111311  111100     -     39
         Intr 111478  111376     -     39
         Intr 111655  111559     -     39
         Init 111850  111750     -     39

45  >6136349          /26930

         Init 126797  126892     +     43
         Term 126964  127308     +     43

50  >6136349          /33975

         Init 129094  129427     +     46
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         Intr 130112  130222     +     46
55       Intr 130626  -130667    +     46
         Term 130793  130849     +     46

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60       Term 131074  130997     -     47
         Intr 131128  131085     -     47
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	Intr	131286	131216	-	47
	Init	131457	131340	-	47

5 >6136349   /254801

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	Term	132880	132932	+	49

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15 >6136350   /37635

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	Intr	5020	4940	-	2
20	Intr	5250	5180	-	2
	Intr	5406	5330	-	2
	Intr	5640	5512	-	2
	Intr	5983	5903	-	2
	Intr	6309	6168	-	2
25	Intr	6617	6424	-	2
	Intr	6781	6704	-	2
	Intr	6984	6897	-	2
	Init	7526	7471	-	2

30 >6136350   /40037

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	Init	14507	14375	-	6

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	Term	16917	17216	+	8

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50 >6136350   /7409

	Term	34815	34623	-	16
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	Intr	35228	35035	-	16
55	Intr	35659	35435	-	16
	Init	35998	35756	-	16

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	Intr	40013	40126	+	17
	Intr	40200	40274	+	17
5	Intr	40462	40572	+	17
	Intr	40652	40765	+	17
	Intr	40964	41030	+	17
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10	Term	41680	41769	+	17

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25	Intr	58438	58257	−	22
	Intr	58626	58517	−	22
	Intr	58831	58727	−	22
	Intr	58975	58897	−	22
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30	Intr	59268	59190	−	22
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35

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40	Init	71255	70827	−	26

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	Init	83853	83263	−	32

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60	Intr	85649	85832	+	34
	Intr	85914	86023	+	34

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        Intr    86103   86198        +        34
        Intr    86350   86775        +        34
        Intr    86861   87016        +        34
        Intr    87109   87323        +        34
 5      Intr    87415   87460        +        34
        Term    87475   87564        +        34

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        Init    88532   88291        -        35

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        Intr    97216   97083        -        41
25      Intr    97365   97296        -        41
        Intr    97631   97567        -        41
        Intr    97804   97767        -        41
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45
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        Intr    20948   20892        -        13
50      Intr    21316   21193        -        13
        Intr    21661   21591        -        13
        Intr    21811   21743        -        13
        Intr    22211   22072        -        13
        Intr    22400   22298        -        13
55      Intr    22606   22484        -        13
        Intr    22759   22683        -        13
        Intr    22987   22842        -        13
        Init    23227   23079        -        13

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         Intr   34395   34288      -      18
 10   Intr   34529   34481      -      18
         Intr   35015   34951      -      18
         Init   35176   35128      -      18

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 15
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         Intr   49921   49832      -      24
         Intr   50548   50450      -      24
 20   Intr   50740   50740      -      24
         Intr   51535   51423      -      24
         Intr   51869   51796      -      24
         Init   52198   51952      -      24

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         Init   59902   60022      +      27
         Intr   60134   60329      +      27
         Intr   60929   60966      +      27
 30   Term   61042   61088      +      27

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 35   Intr   83402   83535      +      33
         Intr   83632   83719      +      33
         Intr   83848   83997      +      33
         Intr   84243   84374      +      33
         Intr   84471   84621      +      33
 40   Intr   84707   84745      +      33
         Intr   84831   84905      +      33
         Intr   85058   85117      +      33
         Intr   85248   85312      +      33
         Intr   85430   85519      +      33
 45   Term   85616   85883      +      33


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	Term	7064	6943	-	6
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	Intr	7554	7451	-	6
	Intr	7847	7633	-	6
10	Intr	8189	7932	-	6
	Intr	8604	8323	-	6
	Intr	9140	8972	-	6
	Intr	9376	9232	-	6
	Init	10134	9984	-	6

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	Term	10748	10524	-	7
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20	Intr	11165	11046	-	7
	Intr	11666	11475	-	7
	Intr	12105	12007	-	7
	Intr	12323	12176	-	7
	Init	12772	12564	-	7

25 >6136352   /108207

	Term	25826	25335	-	13
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30 | Init | 26429 | 26278 | - | 13 |

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35	Intr	27495	27328	-	14
	Intr	27662	27599	-	14
	Intr	27869	27765	-	14
	Intr	28172	28063	-	14
	Intr	28550	28254	-	14
40	Intr	28734	28657	-	14
	Intr	29094	29054	-	14
	Intr	29411	29282	-	14
	Init	30052	29513	-	14

45 >6136352   /40928

	Init	51873	51896	+	19
	Intr	51970	51975	+	19
	Intr	51986	52031	+	19
50	Intr	52264	52355	+	19
	Intr	52456	52536	+	19
	Intr	52657	52754	+	19
	Intr	52837	52917	+	19
	Term	53178	53310	+	19

55 >6136352   /20549

	Init	54814	54900	+	21
	Intr	55020	55085	+	21
60 | Intr | 55164 | 55238 | + | 21 |

109

						109
	Intr	55393	55534	+	21
	Intr	55618	55664	+	21
	Intr	55749	55806	+	21
	Intr	55943	55992	+	21
5	Intr	56076	56134	+	21
	Intr	56404	56514	+	21
	Intr	56604	56688	+	21
	Intr	56796	56862	+	21
	Term	56955	57076	+	21

10

>6136352   /113400

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	Intr	63390	63474	+	26
15	Intr	63866	63984	+	26
	Term	64069	64419	+	26

>6136352   /155785

20	Init	76037	76603	+	31
	Intr	76642	76676	+	31
	Intr	78525	78566	+	31
	Term	78606	78654	+	31

25

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule comprising:

   a) a nucleic acid having a nucleotide sequence which encodes an amino acid sequence exhibiting at least 40% sequence identity to an amino acid sequence encoded by

   (1) a nucleotide sequence described in Tables 1 and/or 2 or a fragment thereof; or

   5
   (2) a complement of a nucleotide sequence shown in Tables 1 and/or 2 or a fragment thereof;

   b) a nucleic acid which is the reverse of the nucleotide sequence according to subparagraph (a), such that the reverse nucleotide sequence has a sequence order which is the reverse of the sequence order of the nucleotide sequence according to

   10
   subparagraph (a);

   c) a nucleic acid capable of hybridizing to a nucleic acid having a sequence selected from the group consisting of:

   (1) a nucleotide sequence which is shown in Tables 1 and/or 2; and

   (2) a nucleotide sequence which is complementary to a nucleotide sequence shown in

   15
   Tables 1 and/or 2, under conditions that permit formation of a nucleic acid duplex at a temperature from about 40°C and 48°C below the melting temperature of the nucleic acid duplex.

2. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to

   a) a nucleotide sequence shown in Tables 1 and/or 2 or a fragment thereof; or

   b) a complement of a nucleotide sequence described in Tables 1 and/or 2 or a fragment

   5
   thereof.

3. The nucleic acid molecule according to claim 1, wherein said nucleic acid comprises an open reading frame.

4. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid is capable of functioning as a promoter, a 3' end termination sequence, an untranslated region (UTR), or as a regulatory sequence.

5. The isolated nucleic acid molecule of claim 4, wherein (a) when said nucleic acid is a promoter it comprises a sequence selected from the group consisting of a TATA box sequence, a CAAT box sequence, a motif of GCAATCG or any transcription-factor

binding sequence, and any combination thereof; and (b) when said nucleic acid sequence is a regulatory sequence it is capable of promoting seed-specific expression, embryo-specific expression, ovule-specific expression, tapetum-specific expression or root-specific expression of a sequence or any combination thereof.

6. A vector construct comprising:

    a) a first nucleic acid having a regulatory sequence capable of causing transcription and/or translation; and

    b) a second nucleic acid having the sequence of the isolated nucleic acid molecule according to claim 1;

wherein said first and second nucleic acids are operably linked and

wherein said second nucleic acid is heterologous to any element in said vector construct.

7. The vector construct according to claim 6, wherein said first nucleic acid is native to said second nucleic acid.

8. The vector construct according to claim 6, wherein said first nucleic acid is heterologous to said second nucleic acid.

9. A host cell comprising an isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is flanked by exogenous sequence.

10. A host cell comprising a vector construct of claim 6.

11. An isolated polypeptide comprising an amino acid sequence

    a) exhibiting at least 40%, or 75%, or 85%, or 90% sequence identity of an amino acid sequence encoded by a sequence shown in Tables 1 and/or 2 or a fragment thereof; and

    b) capable of exhibiting at least one of the biological activities of the polypeptide encoded by said nucleotide sequence shown in Tables 1 and/or 2 or a fragment thereof.

12. An antibody capable of binding the isolated polypeptide of claim 11.

13. A method of introducing an isolated nucleic acid into a host cell comprising:

    a) providing an isolated nucleic acid molecule according to claim 1; and

    b) contacting said isolated nucleic with said host cell under conditions that permit insertion of said nucleic acid into said host cell.

14. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 6.

15. A method of modulating transcription and/or translation of a nucleic acid in a host cell comprising:

a) providing the host cell of claim 9; and

b) culturing said host cell under conditions that permit transcription or translation.

16. A method for detecting a nucleic acid in a sample which comprises:

    a) providing an isolated nucleic acid molecule according to claim 1;

    b) contacting said isolated nucleic acid molecule with a sample under conditions which permit a comparison of the sequence of said isolated nucleic acid molecule with the sequence of DNA in said sample; and

    c) analyzing the result of said comparison.

17. A plant or cell of a plant which comprises a nucleic acid molecule according to claim 1 which is exogenous or heterologous to said plant or plant cell.
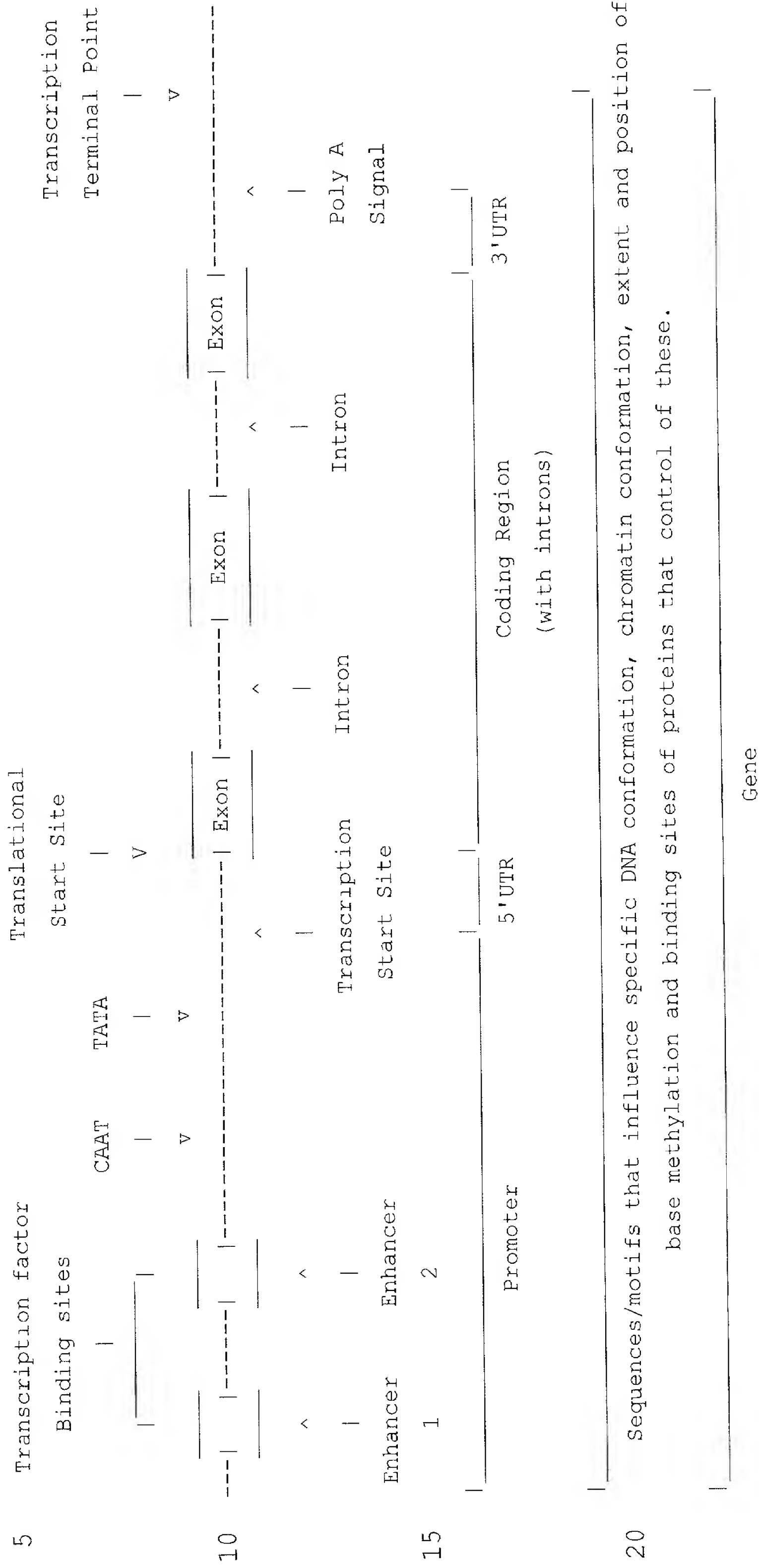
18. A plant or cell of a plant which comprises a vector construct according to claim 6.

19. A plant which has been regenerated from a plant cell according to claim 17.

20. A plant which has been regenerated from a plant cell according to claim 18.

SCHEMATIC 1

SCHEMATIC OF A GENE

Transcription factor
Binding sites

Translational
Start Site

Transcription
Terminal Point

CAAT   TATA

Exon   Exon   Exon

Transcription
Start Site

Poly A
Signal

Enhancer   Enhancer
   1   2

Intron   Intron

5'UTR   3'UTR

Promoter   Coding Region
                   (with introns)

Sequences/motifs that influence specific DNA conformation, chromatin conformation, extent and position of base methylation and binding sites of proteins that control of these.

Gene

ABSTRACT OF THE DISCLOSURE

The present invention provides DNA molecules that constitute fragments of the genome of a plant, and polypeptides encoded thereby. The DNA molecules are useful for specifying a gene product in cells, either as a promoter or as a protein coding sequence or as an UTR or as a 3' termination sequence, and are also useful in controlling the behavior of a gene in the chromosome, in controlling the expression of a gene or as tools for genetic mapping, recognizing or isolating identical or related DNA fragments, or identification of a particular individual organism, or for clustering of a group of organisms with a common trait.

POWER OF ATTORNEY

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Malibu, CA 90265

I, Richard Hamilton, Chief Financial Officer of CERES, INC. of 3007 Malibu Canyon Road, Malibu, California 90265, grant Power of Attorney and authority to empower the following attorneys to act on behalf of CERES, INC. for executing Verified Statements (Declarations) Claiming Small Entity Status to be submitted to the U.S. Patent and Trademark Office in connection with the filing of provisional or regular patent applications on behalf of CERES, INC.

Raymond C. Stewart (Reg. No. 21,066)
Joseph A. Kolasch (Reg. No. 22,463)
Leonard R. Svensson (Reg. No. 30,330)
Gerald M. Murphy, Jr. (Reg. No.28,977)
Mark J. Nuell (Reg. No. 36,623)

This Power of Attorney is to remain in full force and effect until terminated by an official of CERES, INC.

By _____
            Richard Hamilton

Date _____9/24/98_____